ANALYTICAL AND STRUCTURAL STUDIES OF PLANT POLYSACCHARIDES

by

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TO MY MOTHER, HUSBAND AND CHILDREN

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ABSTRACT

The studies presented in this thesis fall into three distinct groups.

a) Analytical characterizations have been made of the gum exudates, not hitherto investigated, from 3 species of the genus Parkia, 6 species of the genus Grevillea, 9 species of the Series Phyllodineae and Gummiferae of the genus Acacia, and 15 specimens of gum obtained from Acacia karroo growing in different locations in Africa. Several of the new species of gum have exceptional features e.g. the intrinsic viscosities of the gums from Grevillea candelabroides and Grevillea wickhamii (521 and 575 $ml g^{-1}$ respectively) and the nitrogen content (7.5%) of A. dictyophleba gum are the highest values reported to date for these parameters in plant gums.

b) A study of some of the structural features of Acacia xanthophloea gum by Smith-degradation, methylation and acid hydrolysis showed the complex acid heteropolysaccharide to be based on a highly-branched β - 1,3 - linked galactose framework which also contained some β - 1,6 - linkages. Glucuronic acid and rhamnose were present as end-groups; arabinose was present in short side-chains, up to three units long, attached to the branched galactan core.

c) The applicability of natural abundance carbon - 13 nuclear magnetic resonance spectroscopy to structural studies of complex acid heteropolysaccharides of high molecular weight was evaluated by examining the sequence of progressively simpler spectra given by the whole gum and its different degradation products.

A preliminary study of the applicability of proton magnetic resonance spectra to complex gum molecules showed that an indication of the amounts of different sugar components present and a rapid non-destructive evaluation of the degree of structural similarity of different gum samples can be obtained.

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SECTION I

GENERAL INTRODUCTION

Plant gums are the most complex of the polysaccharide group of natural products; their study presents the most formidable problem in carbohydrate chemistry (1, 2).

Plant gums are complex acidic polysaccharides exuded from the stems of certain tropical and subtropical trees found in Africa, Australia, India, South America and parts of Asia, after mechanical injury or bacterial infection (3). There is no agreement as to the origin of gum exudates. Some subscribe to the theory (4, 5) that they are a product of normal metabolism, while others suggest that they arise from a pathological condition. Some evidence favours the latter view, for it has been recognized that healthy Acacia trees, grown under favourable conditions of moisture, soil and temperature do not produce any gum, but when they are grown under adverse conditions arising from high elevation, heat and lack of moisture, the secretion of gum is favoured (6). Gum is formed spontaneously after mechanical injury of the tree, by removal of bark or of a branch, or after invasion by insects, bacteria, or fungi. The gums appear to be produced by some protective mechanism in order to seal off the injured portion from attack by organisms or to prevent moisture loss from the wound.

The origin of the gum is also obscure. In early work, it was suggested that gum arises from starch granules present in the cells of the tree. These granules disappear and may be converted into gum which is exuded (3). Theories of the origin, function, precursors, and the mode of biosynthesis of gum exudates have been examined critically (7). The enzyme

systems necessary to transform starch (a polyglucan) into a highly branched heteropolymer of galactose, arabinose, rhamnose, glucuronic acid, and 4-O-methylglucuronic acid appear to be impossibly complex. The mobilisation of hemicellulosic-type arabino-galactans by enzymic modification would be a much more simple biosynthetic route. It is not necessary botanically to propose that invasion by bacteria or fungi is involved as the composition, properties and structure of the exudate from A. senegal appears to be independent of the nature of the wound or stimulus inducing gum production. The ingress of oxygen and the associated physiological disturbance at the active sites of cell growth in the cambium could initiate gum-flow; plant gums seal off wounds and form a protection against complete local dehydration of tissue (7).

The plant gums are usually half-neutralised mixed salts of complex polysaccharide acids, composed of hexose residues, uronic acid residues, pentose and methylpentose residues, all of which are joined together in the most diverse possible ways within the same molecule. All the plant gum polysaccharides studied so far are highly branched, containing more than one type of monosaccharide unit. Around 100 different plant gums from a variety of botanical genera have been studied; xylose and mannose have been found in a few cases, for example in species from the genera Combretum and Grevillea; galactose, arabinose, rhamnose, glucuronic acid (or its 4-O-methyl ether) and galacturonic acid are more frequently involved (8), and glucose has been found in Anacardium occidentale (9).

Exudate gums are generally soluble or partially soluble in water. The use of water soluble gums dates back thousands of years; the Egyptians used gum as paint thickeners and for embalming purposes. Today gums have many varied uses e.g. in the food industry (10) as binding agents in cereal and meat products; as emulsifiers and stabilizers in various oil-in-water emulsions; in confectionery; in wines, beer and soft drinks; in printing inks, lithographic preparations, ceramic glazes, foundry work, etc.

A number of analytical parameters can be used to express the chemical composition and physical properties of a gum. The analytical parameters used to characterize gums are the ash content, nitrogen content, methoxyl content, specific rotation, intrinsic viscosity, molecular weight, equivalent weight, and the ratios of sugars present after hydrolysis. The analytical parameters taken overall, establish a form of "finger print" which serves to characterize each particular species and it now appears that the combined analytical parameters of a plant gum from any genus (Acacia, Albizia, Araucaria, Combretum, Grevillea, Parkia, etc.) give one of the most sensitive ways of establishing the identity of a species (11).

Some gum species give highly viscous solutions and this property makes them very useful commercially. The viscosity of Acacia gums is within the range 4.2 to 27.7ml/g (12), although the work on Grevillea species reported for the first time in this thesis (Section IV) shows that gums of that genus can have viscosities of over 300, a range of values also attained by some Combretum species. The molecular weight of plant gums are

normally within the range 5×10^4 to 3×10^6, although the work on Grevillea and on Parkia species reported in this study (Section III) shows that some gums have molecular weights over 3×10^6.

The acidity of plant gums most frequently arises from the presence of D-glucuronic acid and its 4-O-methyl derivative, but certain gums also contain D-galacturonic acid. The uronic acid content varies considerably from gum to gum and geographical and seasonal variations occur in all the analytical parameters for each species. Typical values for the uronic acid content fall in the range of 10-15%. Some Acacia species, Series Phyllodineae, have been found in the present work (Section V) to contain over 30%, and this value has been exceeded by other Acacias and in studies of gums from other genera.

Gums also contain proteinaceous material. In many species the percentage is small (1-2%), but Acacia dictyophleba has been found in this work (Section V) to contain 52% protein, a value much higher than that reported for Neem gum (Azadirachta indica) (13).

Recently a great deal of analytical work has been done to obtain data (14-16) to support chemical plant taxonomy (17) specially in the genus Acacia which is the most studied of all the gum bearing genera and the most important commercially. Bentham has classified the genus Acacia into six Series (18). Section V of this thesis consists of a detailed analytical study of six previously unstudied Acacia species from Bentham's Series 1 (Phyllodineae). Section VI of this thesis reports work on gums of Bentham's Series 4 (Gummiferae). Subsection A gives analytical

data of 10 samples from different species, and Subsection B gives a comparison of 15 different samples from Acacia karroo obtained from different locations in Africa. Section VII of this thesis consists of a detailed structural study of Acacia xanthophloeia, and Section VIII reports on the use of natural abundance carbon-13 nuclear magnetic resonance spectroscopy as an analytical tool for the study of gum polysaccharides and their degradation products. The final section of this thesis reports some preliminary results from the application of proton magnetic resonance spectroscopy to the analysis and structural elucidation of gum exudates.

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SECTION II

EXPERIMENTAL METHODS

II. 1. GENERAL METHODS

Weighings. All accurate weighings were made within the range of the graticule scale (range, 0-100mg) of a Stanton Unimatic Model C.L.I single-pan balance, having an accuracy of ±0.1mg.

Dialyses of polysaccharides, to remove low molecular weight material, were carried out in cellophane tubing (Kalle Aktiengesellschaft, Wiesbaden) against running tap water for 48-72 hours unless otherwise stated.

Electrodialyses of polysaccharides were carried out in a three-compartment perspex cell fitted with cellophane membranes. The water in the outer electrode compartments was changed regularly to prevent overheating. Electrodialysis was continued until a current (applied voltage = 300 volts) ceased to flow.

Reductions in volume were carried out on a rotary evaporator at temperatures below 40°C.

Moisture contents were determined by heating to constant weight at 105°C.

Ash contents were determined by heating to constant weight in a muffle furnace at 550°C.

Carbon, Hydrogen and Nitrogen contents were determined with a Perkin-Elmer 240 Elemental Analyser.

Methoxyl contents were determined by a vapour phase infrared method (1,2); a calibration curve was based on known weights of methyl iodide.

Equivalent weight determinations on exhaustively electrodialysed polysaccharides were carried out by direct titration with standard sodium hydroxide solution (ca. 0.01N).

Uronic acid contents were calculated from the equivalent weights as (17600/E.W.) i.e. - Values are expressed as uronic anhydride.

Quantitative determinations of sugars.

1. Sugars were separated from hydrolysates by chromatography on previously washed Whatman 3MM papers. After elution from the paper, sugars were estimated, colourimetrically, by the Phenol-sulphuric acid method (3). The optical density was read on a Unicam SP 1300 spectrophotometer using filter 2. Calibration curves were obtained from known weights of sugars.

2. Sugars were determined by gas-liquid chromatography of their alditol acetates on a 3% ECNSS-M column at 190°C (4). The hydrolysate was reduced with sodium borohydride for 3 hours; excess borohydride was then neutralised with acetic acid and the mixture was deionised with Amberlite IR-120(H) resin. After filtration, the mixture was taken to dryness and the residue heated under reflux for four hours with a mixture containing acetic anhydride and pyridine (1:1, v/v). The solution was then cooled and injected into the chromatograph.

II.2. PHYSICAL METHODS

Specific rotations of aqueous and chloroform solutions were measured using the sodium D-line with a Perkin-Elmer Model 141 polarimeter at 20±2°C.

Viscosity determinations were carried out in M-sodium chloride solution in an Ubbelohde suspended-level dilution viscometer at 25.0±0.1°C. A sample of the gum (100-200mg) was dissolved in 1M sodium chloride solution (10ml). The solution

was filtered carefully, before addition to the viscometer. Flow times were measured to within 0.1 sec. by means of a stop watch. The isoionic dilution method was used; the gum solution (6ml) was placed in the viscometer and the flow time was measured. Flow times were also obtained for successive dilutions with M-sodium chloride solutions (four additions of 2ml each). Since preliminary experiments had indicated that any loss of gum from M-sodium chloride solution on filtering was negligible, concentration values were estimated from the dry weight of the gum dissolved in a known volume.

Assuming the densities of M-sodium chloride and gum solutions to be equal for low concentrations of gum, the viscosity number $[\eta]$ is given by:

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c} = \lim_{c \to 0} \frac{t-t_0}{ct_0}$$

where c is the concentration of gum (g/ml), and t_0 and t are the flow times (sec.) for solvent and solution respectively.

Extrapolation of the linear plot of $\frac{t-t_0}{ct_0}$ vs c to c = 0 gives $[\eta]$.

Light scattering measurements were carried out at 27° ± 0.5°C with a SOFICA photogoniodiffusometer Model 4200. Unpolarised green light (546nm.) was selected from a mercury lamp spectrum with a Wratten Kodak No. 61 filter. Using the limiting viscosity number as a guideline to the desirable concentration and using M-sodium chloride solution as solvent, gum solutions were accurately prepared (0.1 - 0.3g in 50 ml). Dilutions of this solution were made; the molecular weight was calculated as an average of three of these solutions. Solutions

were clarified and made dust-free by passage through filters of average pore size 0.80μm (Millipore Ltd., Bedford, Mass., U.S.A.) and then through filters of average pore size 0.22μm with a stainless steel filter holder attached to a 20ml syringe. Concentrations of gum solutions were assumed to be unaltered by ultrafiltration (5).

For each concentration, the intensity of scattered light at various angles between 30° and 150° was recorded and corrected, and corrected scale readings I_θ for angle θ were calculated (6) from the equation:

$$I_\theta = \frac{(\text{Isoln} - \text{Isol}) \sin\theta}{\text{I} + \cos^2\theta}$$

where Isoln and Isol are the scale readings for polymer solution and solvent respectively. The reciprocal corrected scale reading $1/I_o$ is plotted against $\sin^2\theta/2$. Extrapolation of the linear portion of this graph to $\theta = o$ gives a value for $\left[1/I_o\right]_{\theta=o}$. The downward curvature of these graphs at low angles is thought to be caused by dust particles suspended in solution (7).

Molecular weights are found from the equation:

$$M = \frac{R}{\frac{2\pi^2 n_o^2}{\lambda^4 N} \cdot \left[dn/dc\right]^2 \cdot I_B \cdot C \cdot \left[1/I_o\right]_{\theta=o}}$$

where

n_o = refractive index of solvent (1.340)

n = refractive index of solution

N = Avogadro's number (6.023×10^{23})

λ = wavelength of incident light (546nm = 5.46×10^{-5}cm)

C = concentration in g/ml

I_B = intensity diffused, selected for standard benzene (0.5)

dn/dc = refractive index increment

R = Rayleigh constant (16.3×10^{-6})

Using the dn/dc value of 0.146, which is the average value found (8) for a series of Acacia gums, the equation can be simplified to:

$$M = \frac{2.309 \times 10^2}{C\,[1/I_o]\,\theta=o}$$

Infrared spectroscopy was carried out with a Hilger-Watts H.1200 double beam grating spectrophotometer.

Ultracentrifugation was carried out with a Beckman-Spinco Model E Analytical Ultracentrifuge. Polysaccharide solutions (0.5% in 0.5M sodium chloride solution) were examined at 44,000 r.p.m. After the ultracentrifuge had attained this speed, the boundary patterns, obtained by a Schlieren optical system, were photographed at 16,8 or 4 min intervals.

Proton n.m.r. spectroscopy . N.m.r. spectra were obtained at 100 MHz with a Varian XL-100 n.m.r. spectrometer equipped with Fourier Transform facilities. Samples of gum (5-10mg) were dissolved in deuterium oxide (1ml). The spectrometer was field-frequency locked to the deuterium in the D_2O solvent. An inversion recovery sequence method, (9,10) which eliminates the residual HDO signal, was used to obtain the spectra.

Carbon-13 n.m.r. spectroscopy. Spectra were recorded with a Varian CFT-20 Carbon-13 nuclear magnetic resonance spectrometer. Data points were accumulated overnight at a temperature of 36°C, a spin rate of 22 r.p.s. and with complete proton decoupling. The number of transients varied from 150,000 to 280,000 depending on the quantity of material available. The spectral width was 4000 Hz

(200p.p.m.) and the spectra were calibrated by the addition of small amounts of 1,4-dioxan to the samples. Samples of polysaccharides (100-200mg) were dissolved in deuterium oxide (1ml).

II. 3. CHEMICAL METHODS

Small scale polysaccharide hydrolyses were carried out with 0.5M sulphuric acid for 8 hours on a boiling water bath, unless otherwise stated. Hydrolysates were neutralised with barium carbonate, filtered, deionised with Amberlite IR-120(H) resin, and concentrated on a rotary evaporator.

Small scale polysaccharide methylations.

a) The Haworth method (11)

Methylations were carried out in an atmosphere of nitrogen at room temperature. Dimethyl sulphate (2ml) and sodium hydroxide (2ml, 30% (W/V), were added dropwise with stirring to the polysaccharide (100-500mg) in water (10ml) over a period of one hour. Acetone (5ml) was added to the reaction mixture and six further additions of dimethyl sulphate (12ml) and sodium hydroxide (17ml) were made, allowing three hours for each addition. After stirring for twelve hours, the reaction mixture was heated at 60°C for 30 minutes with nitrogen bubbling vigorously through the solution. After cooling, the reaction mixture was neutralised with 2M-sulphuric acid and made slightly acid (pH = 4.0); a white precipitate was normally observed at this stage. The methylated product was extracted into chloroform (4 x 100 ml extractions) and the extract was shaken with saturated sodium chloride solution (ca. 100ml). The chloroform layer was separated, dried over anhydrous sodium sulphate, and concentrated on a rotary evaporator. The concentrated syrup was poured into light

petroleum (b.p. 60°-80°, ca. 400ml) with stirring; the precipitated methylated polysaccharide was isolated after filtration and drying, as a white amorphous powder.

b) The Purdie and Irvine method (12)

The partially methylated polysaccharide (100-400mg) was dissolved in methanol (5ml) and methyl iodide (10ml). Silver oxide (1g) was added in four batches of ca. 250mg. every 1.5 hours; the mixture was heated under reflux for 6 hours in darkness in a dry flask fitted with a water condenser and a calcium chloride tube. The mixture was cooled and filtered through sintered glass and the residue was extracted six times with hot chloroform (ca. 50ml). The combined filtrate and extracts were reduced in volume and any dissolved silver ions were removed by passing hydrogen sulphide through the solution and refiltering. After concentration to small volume, the syrup was poured into light petroleum (b.p. 60°-80°, ca. 400ml) with stirring. After filtration and drying, the precipitated methylated polysaccharide was isolated as a white amorphous powder.

Small-scale oligosaccharide methylations (13,14). The oligosaccharide (0.5-2.0mg) was shaken with methyl iodide (0.2ml) and silver oxide (0.2g) at room temperature in darkness for 18 hours. The mixture was filtered and the residue washed with chloroform. The combined filtrate and washings were concentrated to a syrup on a rotary evaporator.

Methanolyses were carried out under reflux for 6 hours with methanolic 5% hydrogen chloride. Solutions were cooled and were taken to dryness in a vacuum desiccator filled with calcium chloride and containing a few pellets of potassium hydroxide. The residue was taken up in chloroform and concentrated to a small volume.

Periodate oxidations of polysaccharides were carried out in

darkness at room temperature.

a) Consumption of periodate. The amount of periodate consumed by a polysaccharide was estimated by back-titration of the excess of periodate. An excess of potassium iodide was added to a portion (1ml) of the periodate solution, and the iodine liberated was titrated, after the addition of sodium bicarbonate (200mg), with standard sodium arsenite solution (ca. 0.025M) using "Thyodene" as indicator (15).

b) The formic acid released was estimated titrimetrically (16) with standard sodium hydroxide (ca. 0.1M) for portions (1ml) of the solution. Methyl red was used as the indicator.

II. 4. CHROMATOGRAPHIC SEPARATIONS

Paper chromatography of sugars was carried out on Whatman No. 1 papers, unless otherwise stated, with the following solvent systems (v/v):

(a) ethyl acetate, acetic acid, formic acid, water (18:3:1:4)

(b) benzene, butan-1-ol, pyridine, water (1:5:3:3, upper layer)

(c) ethanol, phosphoric acid (0.1N), butan-1-ol (10:5:1) (17)

(d) butan-1-ol, ethanol, water (4:1:5, upper layer)

Before using solvent (c) papers were dipped in 0.3M-sodium dihydrogen orthophosphate solution and air dried.

Reducing sugars were detected by spraying chromatograms with a saturated solution of aniline oxalate in ethanol, water 1:1 (v/v); then heating at 150°C for ca. 3 minutes, R_{gal} values of sugars refer to the distance moved relative to that of D-galactose. R_g values of O-methyl sugars refer to distances moved relative to that of 2,3,4,6-tetra-O-methyl-D-glucose.

Gas liquid partition chromatography (g.l.c.) of mixtures of O-methyl sugars was carried out with a Pye 104 chromatograph at nitrogen flow rates of 25 ml/min. This instrument was fitted with a flame ionisation detector. The column (200 x 0.3cm) used was 15% by weight of polyethylene glycol adipate on Universal B (phase-sep) at 170°C. Retention times (T) are quoted relative to methyl 2,3,4,6 - tetra - O - methyl - β - D - glucopyranoside as standard.

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SECTION III

A STUDY OF THREE *PARKIA* GUMS

III.1. INTRODUCTION

Parkia is a circumtropical genus comprising about 40 species (1). Several species occur in tropical America and tropical Asia; the four species known to occur in Africa (P. africana, P.biglobosa, P. bicolor and P. filicoidea) are widespread and somewhat variable. An introduced and rarely grown species (P. roxburghii G. Don) also occurs in Africa (2).

Parkia is a natural taxon, immediately distinguishable in MIMOSACEAE by its remarkable pendent double inflorescences and valvate calyx-lobes. The African species are closely allied. Seed-characters are decisive in distinguishing between P. biglobosa, P. bicolor and P. filicoidea. The seeds of P. biglobosa and P. africana, however, are similar.

R. Brown (3) proposed the genus for the first time. G. Bentham (4) made a revision of the Sub-order Mimoseae and his classification of the genus Parkia is given in Table III.1.

So far, studies have not been made on any Parkia gum exudates. Studies of the seeds of P. Filicoidea have included the determination of their nutritional value (5) and of their fatty acids (6). The determination of the protein value of seeds of Nigeria (7) has included P. biglobosa and P. africana. A chemical study of the fruit pulp and seeds of P. biglobosa (8) has been made.

This section of this thesis presents the results of an analytical study of the gums from Parkia pendula (a tropical American species) and from Parkia bicolor and Parkia biglobosa, which are African species.

TABLE III.1.

Bentham's Classification of the genus Parkia

Tribe 1 Parkieae, genus 2 Parkia

Section 1 EUPARKIA

1.	P. biglandulosa, W. et Arn.	East Tropical Asia
2.	P. roxburghii, G. Don	Tropical Asia
3.	P. leiophylla, S. Kurz	" "
4.	P. macrocarpa	" "
5.	P. insignis, S. Kurz	" "
6.	P. singularis	" "
7.	P. africana, R. Br.	Tropical Africa
	P. biglobosa, Benth.	" "
8.	P. intermedia Oliv.	" "
9.	P. filicoidea, Welw. in Oliv.	" "
10.	P. platycephala, Benth.	Tropical America
11.	P. pendula, Benth.	" "
12.	P.multijuga Benth.	" "

TABLE III.1.(continuation)

Genus 2 *Parkia*

Section 2 PARYPHOSPHAERA

13.	*P. pectinata* Benth.	Tropical	America
14.	*P. filicina* Benth.	"	"
15.	*P. auriculata* Spruce	"	"
16.	*P. discolor* Spruce	"	"
17.	*P. nitida*	"	"
18.	*P. paryphosphaera* Benth.	"	"
19.	*P. oppositifolia* Spruce	"	"

III.2. ORIGIN OF GUM SAMPLES

Seed pods from *Parkia pendula* Willd. Benth., collected by Prof. D.H. Jansen, Botany Dept., University of Philadelphia, in Corcovado National Park, Puntarenas Prov., Costa Rica, on 20 March, 1977, contained seeds surrounded with a thick coating of gum.

Gums exuded from *Parkia bicolor* and *Parkia biglobosa* were collected by Mrs. H.C. Hopkins, Botany Dept., University of Oxford, at Forest Research Institute, Ibadan, Nigeria, on 10 February 1978.

III.3. PURIFICATION OF SAMPLES

Crude gums (1-2g where available) were dissolved in distilled water (ca. 100ml). After 2 days, the solutions were filtered through Whatman No. 1 and No. 42 filter papers and dialysed against running tap water for 2 days. The polysaccharides were obtained as the freeze-dried products.

In the case of *Parkia pendula* the seeds with their coating of gum were placed in water; after the gum had dissolved, the seeds were removed by filtration.

III.4. ANALYTICAL DATA

The analytical data for the three *Parkia* samples are given in Table III.1.

The samples were hydrolysed with 0.5M sulphuric acid and the hydrolysates were examined by paper chromatography in solvents (a) and (b). The gums all contained galactose and arabinose. Rhamnose was not detected chromatographically. The gums also contained two aldobiuronic acids (R_{gal} 0.2 and 0.67 in solvent (b) which

corresponded chromatographically to 6-O(β-D-glucopyranosyl uronic acid)-D-galactose and 4-O(4-O-methyl-α-D-glucopyranosyl uronic acid)-D-galactose, which are the most commonly occurring aldobiuronic acids in Acacia gums (9).

The Parkia gums studied have very high molecular weights. Parkia biglobosa gum has a molecular weight (3.2×10^6) that is comparable to that reported for the gums from Acacia holosericea and Acacia mangium and which is regarded as the highest molecular weight reported (10) for an Acacia gum so far. Parkia pendula gum has a molecular weight (5.6×10^6) that is even higher than that of the gums from some Grevillea species (See Section IV).

The Parkia species studied gave gum solutions of relatively high viscosity. Although the values are lower than those obtained for gums from some Grevillea species (See Section IV), and from Combretum collinum gum (12), they are similar to the viscosity of Acacia saliciformis gum which is regarded as the most viscous Acacia gum studied so far (See Section IV).

The nitrogen content of the Parkia gums is similar to that for many Acacia gums (13) but higher than that for Combretum gums (12) and for Grevillea gums (Section IV).

The methoxyl contents of the Parkia gums are within the range found in many plant gums (14).

The analytical data for the Parkia samples studied reveal that although the African samples (from Parkia bicolor and Parkia biglobosa) are chemically very similar, they differ considerably from the Central American sample (Parkia pendula). The main differences occur in the values obtained for the specific rotations,

TABLE III.1

ANALYTICAL DATA FOR PARKIA GUMS

	Parkia pendula	Parkia bicolor	Parkia biglobosa
Moisture,%	5.63	8.27	9.96
Ash,%[a]	1.5	3.0	3.2
Nitrogen,%[a]	0.35	0.92	0.95
Hence Protein, %[a]	2.2	5.8	5.9
Methoxyl,%[b]	0.72	1.25	1.06
Specific rotation, degrees[b]	-74	+13	+33
Intrinsic Viscosity, ml/g[a]	34	44	32
Molecular weight, [a] $\overline{Mw} \times 10^6$	5.6	2.0	3.0
Equivalent weight	2277	1033	962
Hence uronic acid,%[b,c]	8.0	17.0	18.0
Sugar composition[b] after hydrolysis:-			
4-O-Methylglucuronic acid[d]	4.3	7.5	6.4
Glucuronic acid	3.7	9.5	11.6
Galactose	30	74	73
Arabinose	62	9	9
Rhamnose	-	-	-

[a] = Corrected for Moisture content.

[b] = Corrected for moisture and protein contents.

[c] = If all acidity arises from uronic acids.

[d] = If all methoxyl groups located in this acid.

uronic acid content, and in the sugar composition (See Table III.1). These differences are not surprising and probably do not reflect geographical differences; the gums from Parkia bicolor and Parkia biglobosa were tree exudates whereas the product from Parkia pendula was obtained from within the pods. This unusual form of polysaccharide is believed (15) to act as a form of protection for the seeds, either to protect them from desiccation prior to favourable germination conditions or to protect them from digestive enzymic juices should they be swallowed by animals. In terms of the solubility, physical properties and chemical composition of this acidic polysaccharide, it must be classed as falling within the classification of a plant gum.

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SECTION IV

A STUDY OF GUM EXUDATES FROM THE GENUS *GREVILLEA*

IV. 1. INTRODUCTION

The Proteaceae comprise evergreen trees, shrubs and undershrubs and, rarely, perennial herbs.

Robert Brown (1) was the first botanist to study this interesting family and to classify it into sub-families and tribes. On the basis of the fruit characters, Brown (1) divided the Proteaceae into two sub-families; the Nucamentaceae (fruits indehiscent) and Folliculares (fruits dehiscent). Later Engler (2) changed these names into Persoonioideae and Grevilloideae respectively. The name Persoonioideae has now been altered to Proteoideae in accordance with the international rules of botanical nomenclature, because Protea (136 species) is the largest genus of this sub-family; the name Grevilloideae has been retained as Grevillea (235 species) is the largest genus of the second sub-family.

Brown (1) divided the Proteoideae into four tribes, Persoonieae, Franklandieae, Proteeae and Conospermeae and the Grevilleoideae into three tribes, the Grevilleae, Embothrieae and Banksieae. Bentham (3) accepted Brown's classification but Engler (2) added another taxonomic criterion for distinguishing the sub-families, viz. the occurrence of flowers singly in bract axils in the Proteoideae and in pairs in the axils of common bracts in the Grevilleoideae. His studies have shown that this is a better criterion than the fruit characters, and Engler (2), like Bentham (3), maintained Brown's tribes.

In the above classification very few taxonomic criteria have been used and even these are not applied consistently (4).

The classification of the Proteaceae was revised by Venkata Rao in 1957 and further revised by the same author in 1971 (4). He has used for this purpose evidence from many sources such as morphology, wood anatomy, floral anatomy, cytology, palynology and embriology.

In 1975 Johnson and Briggs (5) reviewed the classification and 75 genera are now recognized in place of 62. Most of the familiar genera are retained unchanged, but it was concluded that Persoonia, sensu lato, should be divided into four genera (5) as envisaged in an earlier study. In this classification there are new sub-families, tribes, and sub-tribes.

Johnson and Briggs' classification is given in Table IV.1.

The overall present distribution of the Proteaceae is shown on Table IV.2 which summarizes the numbers of genera in each sub-family and tribe found in the three super-regions centred on Australia, Africa and South America.

Chemical features have been discussed in relation to the origins of the Proteaceae but the available data are insufficient for a critical assessment of relationships within the family.

Phenolic compounds in some South African genera were studied by Rheede van Oudtshoorn (6); Vickery (7) compared the fatty acids of the seed oils in 26 species spanning 10 of the tribes now recognized. The former study revealed only slight differences between the genera; the latter tended to give broad support to the present classification.

A chemical difference associated with habitat involves the accumulators and non-accumulators of aluminium on the leaves (8).

TABLE IV.1

TAXONOMIC CLASSIFICATION OF THE FAMILY PROTEACEAE*

SUBFAMILY Tribe Subtribe	No. of genera
PERSOONIOIDEAE	7
Persoonieae	6
Placosperminae	
Persooniinae	
Bellendeneae	1
PROTEOIDEAE	26
Conospermeae	11
Cenarrheninae	
Dilobeinae	
Stirlinginae	
Petrophilinae	
Conosperminae	
Franklandieae	2
Franklandiinae	
Adenanthinae	
Proteeae	13
Aulacinae	
Proteinae	
SPHALMIOIDEAE	1
CARNARVONIOIDEAE	1

* Johnson & Briggs's classification, 1975

TABLE IV.1 (Continuation)

TAXONOMIC CLASSIFICATION OF THE FAMILY PROTEACEAE

SUBFAMILY Tribe Subtribe	No. of genera
GREVILLEOIDEAE	40
Oriteae	2
Knightieae	4
Knightiinae	
Cardwelliinae	
Embothrieae	8
Stenocarpinae	
Buckinghamiinae	
Lomatiinae	
Embothriinae	
Grevilleeae	3
Helicieae	4
Hollandaeinae	
Heliciinae	
Triuniinae	
Macadamieae	15
Hicksbeachiinae	
Gevuininae	
Floydiinae	
Macadamiinae	
Roupalinae	
Lambertiinae	
Banksieae	4
Musgraveinae	
Banksiinae	
Total PROTEACEAE	75

TABLE IV.2 (reproduced from Botanical J. Linn.Soc., 1975,70.)

GENERIC DISTRIBUTION AND ENDEMISM - EXTENDED CONTINENTAL SUPER-REGIONS‡

SUBFAMILIES Tribes	Africa including Madagascar	Australasia, W. Pacific & E. Asia	S. & C. America	Total
PERSOONIOIDEAE	0	***7(7)	0	7
Persoonieae	0	***6(6)s	0	6
Bellendeneae	0	1(1)s	0	1
PROTEOIDEAE	****14(14)	***12(12)	0	26
Conospermeae	1(1)	***10(10)s	0	11
Franklandieae	0	**2(2)s	0	2
Proteeae	****13(13)s	0	0	13
SPHALMIOIDEAE	0	1(1)	0	1
CARNARVONIOIDEAE	0	1(1)	0	1
GREVILLEOIDEAE	2(2)	****34(30)	***8(4)	40
Oriteae	0	*2(1)±s	1(0)±s	2
Knightieae	0	*4(4)	0	4
Embothrieae	0	**7(5)±s	*3(1)±s	8
Grevilleeae	0	****3(3)±s	0	3
Helicieae	0	***4(4)	0	4
Macadamieae	2(2)	**10(9)±s	***4(3)	15
Banksieae	0	***4(4)s	0	4
TOTAL PROTEACEAE	16(16)	55(51)	8(4)	75

‡ The total number of genera is shown for each super-region together with (in parentheses) the number confined to the super-region. Concentrations of species are asterisked (*6-20spp.,**21-50spp.,***51-150spp.,****>150spp. Occurrences where the major diversification is of markedly sclerophyllous or otherwise xeromorphic species are marked "s", or "±s" where the species are somewhat sclerophyllous or where approximately equal numbers are sclerophyllous and non-sclerophyllous.

Chemical analysis offers one of the possible approaches to taxonomy. Unfortunately, the chemical data available at present are limited. Interest in chemotaxonomy is growing quickly; useful correlations have been obtained in a number of plant groups (9-13). Generally the most useful substances for chemical taxonomy belong to the so-called secondary products such as tannins, essential oils, alkaloids, glycosides and gum exudates.

One of the objectives of Acacia gum chemistry is to obtain chemotaxonomic data and to compare the chemical parameters of gums from morphologically similar Acacia trees. Such studies can only be realistic, however, if the botanical origins of the gum samples are accurately known.

Unlike the Acacia genus, in which, extensive work has been carried out in the field of chemical taxonomy, very little is known about the chemistry of gum of the Grevillea genus, which comprises about 170 spp., (14), mostly in Australia, New Caledonia (9 spp.) and Malaysia (4 native spp.).

The only analytical and structural investigations so far have been carried out on the gum from Grevillea robusta (15,16).

A detailed analytical study has now been carried out on the gum exudates from six Grevillea species, e.g. Grevillea robusta, Grevillea candelabroides, Grevillea striata, Grevillea wickhamii (two specimens) and Grevillea agrifolia.

Table IV.3 shows the botanical relationship between the genera and species examined in this study.

TABLE IV.3

Family — Sub-families

PROTEACEAE (order Proteales)

a) PERSOONIOIDEAE

b) PROTEOIDEAE

c) SPHAIMIOIDEAE

d) CARNARVONIOIDEAE

e) GREVILLEOIDEAE (40 genera)

TRIBE
Oriteae
Knightieae
Embothrieae

TRIBE
Grevilleeae
(3 genera)
Hakea
Finschia
Grevillea (170 spp.)
G. robusta
G. candelabroides
G. striata
G. wickhamii
G. agrifolia

TRIBE
Helicieae
Macadamieae
Banksieae

IV.2. ORIGINS OF GUM SPECIMENS

Gum from Grevillea robusta Cunn. ex R. Br. was obtained in 1972 from pruning wounds on a tree growing in the Temperate House, Royal Botanic Garden, Edinburgh.

Gum from Grevillea candelabroides C.A. GARDN. was collected on 11 June 1976 by Mr. D.J. McGillivray at 29°15'S, 115°29'E, 9Km from Mingenew on the road to Three Springs, W. Australia.

Gum from Grevillea striata was collected by Mr. J.R. Maconochie on 22 April 1975, at Alice Springs.

Gum from Grevillea wickhamii (sample 1) was collected by Mr. J.R. Maconochie at Napperby Station, Northern Territory, Australia, on 8 May 1975.

Gum from Grevillea wickhamii (sample 2) was collected by Mr. Maconochie at Napperby Station, N.T., on 21 June 1976.

Gum from Grevillea agrifolia A. Cunn. ex R. Br. was collected by D.J. McGillivray on 23 June 1978 at 16°08'S, 126° 30'E at Gibb river crossing on the road to River Station, Western Australia.

IV.3. PURIFICATION OF GUM SAMPLES

All the gum samples dissolved readily in cold water except that from G. wickhamii (sample 2), which required mild treatment with sodium borohydride for 2 days to facilitate almost-complete dissolution. The solutions were filtered through No. 42 and No. 1 filter papers, dialysed against running tap water for two days (four days in the case of the sample treated with borohydride), refiltered, and freeze dried.

IV.4. ANALYTICAL STUDIES

Each sample (50mg) was hydrolysed with 0.5M sulphuric acid for 24 hours on a boiling water bath. The hydrolysis time (7.5 hours) used for *Acacia* gums was not long enough for *Grevillea* gums. The solutions were neutralised, deionised and concentrated. Paper chromatography in solvents (a) and (b) indicated that the *Grevillea* gums were more complex than those of the genus *Acacia*. The four main sugar components were galactose, arabinose, xylose and rhamnose, and small amounts of mannose were on the chromatograms run in solvent (a).

Each sample (50mg) was hydrolysed with 1M-sulphuric acid for 12 hours on a boiling water bath. After neutralisation, deionisation, and concentration, paper chromatography of the hydrolysates in solvent (c) indicated the neutral sugars found previously together with a brown spot, (R_{gal} 0.75) characteristic of D-glucuronic acid and a reddish brown spot (R_{gal}0.64) characteristic of galacturonic acid (except in the case of *G. agrifolia*).

The presence of both glucuronic acid and galacturonic acid was carefully checked by running the 1M-hydrolysates in solvent (c) against standard D-glucuronic acid and D-galacturonic acid; the distinction was complete and unambiguous, both in terms of the R_{gal} value and spot colour.

The analytical data obtained for the six samples are shown in Table IV.4.

The analytical methods used were as described in Section II, with the exception that the sugar ratios were determined by running each hydrolysate in solvents (a) and (b) rather than in one solvent. Solvent (a) gave the ratio galactose: mannose:

TABLE IV.4

ANALYTICAL DATA FOR THE GUMS FROM GREVILLEA SPECIES

	G.robusta	G. candelabroides	G. striata	G. wickhamii (1)	G. wickhamii (2)	G. agrifolia
Moisture,%	11.0	15.5	7.8	12.4	6.8	11.4
Ash,%	5.8	3.3	1.4	2.6	3.4	2.9
Nitrogen,%[a]	0.20	0.04	0.24	0.07	nil	0.10
Hence Protein,%[a]	1.3	0.25	1.5	0.43	-	0.62
Methoxyl,%[b]	0.68	1.0	0.50	0.77	0.23	0.08
Specific rotation,[b], degrees	-24	+10	-71	-36	-33	-33
Intrinsic Viscosity,[a] ml/g	89	521	26	575	356	312
Molecular weight, [a] $\overline{Mw} \times 10^5$	88	8.6	8.8	23	36	27
Equivalent weight	1296	1030	1921	2000	3645	1957
Hence Uronic acid,%[b,c]	13.6	17.0	9.0	8.8	4.8	9.0
Sugar composition [b] after hydrolysis:-						
4-O-Methylglucuronic acid[d]	4.1	6.0	3.0	4.6	1.3	0.48
Glucuronic acid + galacturonic acid	9.5	11.0	6.0	4.2	3.5	8.52
Galactose	42	56	46	50	53	40
Mannose	4	2	trace	trace	trace	trace
Arabinose	36	16	29	27	23	32
Xylose	-	7	9	14	19	19
Rhamnose	4	trace	7.0	-	-	-

[a] = corrected for moisture content.

[b] = corrected for moisture content and protein content.

[c] = If all acidity arises from uronic acids.

[d] = If all methoxyl groups are located in this acid.

(arabinose + xylose): rhamnose. Solvent (b) gave the ratio galactose: (arabinose + mannose): xylose: rhamnose and hence the relative ratio for each sugar was calculated.

Neutral sugars were also determined by g.l.c. after formation of the alditol acetates.

The calculation of neutral sugars was made on the assumption that all uronic acid residues are attached to galactose.

The percentage of 4-O-methyl-D-glucuronic acid in each sample was calculated on the assumption that all the methoxyl groups are located on the 4-O-methyl-D-glucuronic acid residues.

Molecular weights were calculated using the average value for dn/dc found (17) for a series of *Acacia* gums; a suitable differential refractometer was not available during the period of the present study.

The gums studied have high molecular weights. The molecular weights reported for *Acacia holosericea* (3.8×10^6) and *Acacia mangium* (3.2×10^6) are the highest values reported (18) for *Acacia* gums so far. These values are exceeded by the molecular weight of *Grevillea robusta* gum (8.8×10^6), but a higher molecular weight (14×10^6) has been reported for a member of the genus Combretum (19).

The *Grevillea* species studied gave gum solutions of exceptionally high intrinsic viscosity, with the exception of *Grevillea striata*. The value given for *Grevillea robusta* gum ($[\eta] = 89$ ml/g) is higher than the highest value recorded for *Acacia* gums (See Section VI, Phyllodineae); the viscosities of *Grevillea candelabroides* gum and *Grevillea wickhamii* gum (sample 1) are much higher than the values obtained for *Combretum collinum* (312 ml/g) (19). The gums

from G. candelabroides and G. wickhamii with intrinsic viscosities of 521 and 575 ml/g respectively are the most viscous gums studied so far, and may therefore be of potential commercial interest.

The nitrogen content and hence the protein content of these gums was very low; similar situation has been recorded for Combretum gums (19).

The methoxyl contents, although showing a wide variation, are within the normal range found in plant gums.

The specific rotation also show a wide variation (from -71° (G. striata) to $+10^{\circ}$(G. candelabroides). The highest negative rotation (-81°) recorded so far was that for Combretum collinum (19).

The Grevillea gums have low uronic acid content, with the exception of G. candelabroides gum (uronic acid content = 17.2%).

The sugar ratios (see Table IV.4) show quite wide variation in the ratio of galactose: arabinose: xylose, and some samples also contain mannose and rhamnose. The presence of mannose and xylose have also been reported in Combretum gums (20), but they do not occur in Acacia gums.

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SECTION V

AN ANALYTICAL STUDY OF SOME *ACACIA* GUM EXUDATES OF THE SERIES PHYLLODINEAE

V.1. INTRODUCTION

The genus Acacia is very large and it still provides many complex botanical problems of nomenclature and classification. The number of species in the genus is not known with certainty; Black (1) estimated the number at 500, Brenan (2) and Ewart (3) proposed 750-800 and Hutchinson (4) suggested 900. Tindale listed 616 spp. native to Australia alone. The known indigenous African and American spp. are probably sufficient to bring the total for the genus to at least 900.

The genus Acacia was divided into six Series and fifteen sub-series by Bentham (6) and into Sections and Sub-sections by Taubert (7).

Bentham's divisions, based upon habit, inflorescence and geographical distribution, are: Series 1, Phyllodineae, containing 570 spp. (5), subdivided into 8 sub-series; Series 2, Botryocephalae, 32 spp. (5); Series 3, Pulchellae, 14 spp. (5); Series 4, Gummiferae,60spp. (6), 3 sub-series; Series 5, Vulgares, 75 spp. (6), 4 sub-series; Series 6, Filicinae, 2 spp. (6).

Species from Series 1 are native to Australia, Hawaii and New Caledonia; species from Series 2 and 3 are native to Australia; species from Series 4 and 5 are found throughout tropical and semitropical parts of the world; and species from Series 6 are native to South America.

Although various revisions have been necessary with the discovery of new species, Bentham's main divisions (6) or Series are still used. Recently Vassal (8) proposed a revision of Bentham's divisions, but his proposals have not been accepted universally.

The number of Acacia gum species studied has increased from

13 in 1963, to 30 in 1970 and to 44 in 1972; by 1977 a further 42 different Acacia gums had been studied or were in the process of being studied.

Comparisons of the analytical and structural data for species belonging to Bentham's Series 1, 4 and 5 have been made (10); in general, the chemical evidence substantiates Bentham's taxonomic divisions, and the broad bases of difference shown by gums belonging to the different series in Acacia have been tabulated (10). Gum exudates from Acacia species in Series 3 and 6 of Bentham's classification have not yet been studied.

Series 1, Phyllodineae, comprises at least 570 species; it will be a long time before analytical data for a sufficiently large proportion of these species becomes available for a statistically acceptable evaluation to be possible. The gums of this series (11) appear to be characterized by low molecular weight, low acidity, low positive or negative rotations, low rhamnose, and a high ratio of galactose to arabinose.

Series 2, Botryocephalae, comprises 32 species. The data available at present for 12 species of this Series indicate that it may contain two chemically distinct types. Species of one of these types (type A) differ greatly in composition from gums of the Phyllodineae; the gums from the so-called type B species show strong resemblances to those of the Phyllodineae.

Series 4, Gummiferae, are a predominantly African group of Acacias, and as such, have received more attention than some Australian gums. Gums of this series are characterized mainly by highly positive optical rotations and high molecular weight, with a tendency towards intermediate values of acidity and viscosity and low proportions of rhamnose; wide variations in nitrogen and methoxyl values occur, however.

Series 5, Vulgares, contains the most commercially important Acacia gum, that from A. senegal, which has been the subject of more chemical investigations e.g. into its seasonal and geographical variation, and more detailed structural analyses than the gum from any other Acacia species.

The main features of the gums from these species appear to be significant negative optical rotations, intermediate molecular weights (of the order of 0.5×10^6) and the presence of significant proportions of rhamnose.

Chemical analyses of ca. 90 different species have now been carried out (9,10) with the conclusion that each Acacia species exudes a gum that is characteristic of that species regardless of where it is grown geographically i.e. the chemical composition and physical properties of the exudate from each Acacia species differ, often very considerably, from that of other species.

Relatively few species of the Series Phyllodineae have been examined chemically so far, although there have been studies of the distribution of amino acids in some seeds (12) and of the flavonoid content of some heartwoods (13,14). To date the gum exudates from 25 species (15,16) in the series Phyllodineae have been studied; of these one is in Bentham's sub-series 4C, eleven are in subseries 6F, one is in subseries 7F and 12 species are in sub-series 8 (Juliflorae) (16).

Data for four gums that appear to be typical of the species in subseries 1 - 7 of this Series are given in Table V.1. Table V.2. shows the analytical parameters for some Juliflorae species (sub-series 8).

A detailed analytical study has now been made of the gums from a further six species of the Phyllodineae. The gums studied were from A. saliciformis, A. xanthina and A. rostellifera (which are placed in Bentham's Series 1, subseries 6F) and A. dictyophleba, A. cyclops (which are placed in Bentham's Series 1, subseries 7F). An undescribed species of unknown affinities within Phyllodineae was also studied in an attempt to find the known species to which it corresponds most closely.

V.2. ORIGIN OF GUM SPECIMENS

Gum from Acacia saliciformis Tindale was collected by R. Coveny and P. Hind, on 14 December 1976, 19.7 Km NNW of Colo Heights, N.S.W. Australia. The botanical voucher specimen for this tree has been kindly authenticated by Dr. Tindale as NSW 108003.

Gum from Acacia xanthina Benth. was collected by Mr. B.R. Maslin, Perth, Western Australia, in September 1976; the reference voucher number is BRM 4291.

Gum from Acacia rostellifera Benth. was collected by Mr. B.R. Maslin in W. Australia in September 1975; the reference voucher number is BRM 3817.

Gum from Acacia dictyophleba F. Muell. was collected by Mr. P.K. Latz on 14 May 1975 at Amburla Station, W. Australia.

Gum from Acacia cyclops A. Cunn. ex G. Don was collected by Mr. B.R. Maslin in W. Australia in September 1975; the reference voucher number is BRM 3893.

Gum from an un-named new species was collected by Mr. B.R. Maslin in W. Australia in September 1975; the reference voucher

TABLE V.1

DATA FOR THE GUMS FROM SOME PHYLLODINEAE SPECIES OF ACACIA

	Acacia difformis	Acacia falcata	Acacia mabellae	Acacia retinoides
Ash, %	1.5	1.8	1.7	2.1
Nitrogen,%	0.28	0.21	0.23	0.48
Methoxyl,%	0.64	0.49	0.41	0.41
Specific rotation, degrees	-5	+9	+4	+1
Intrinsic viscosity,ml/g	6.2	5.1	5.8	9.5
Molecular weight, $\bar{M}_w \times 10^3$	47	79	120	730
Equivalent weight	3420	2290	2870	1770
Hence uronic acid, %	5	8	6	10
Sugar composition after hydrolysis:				
4-O-Methylglucuronic acid	3.5	3	2.5	2.5
Glucuronic acid	1.5	5	3.5	7.5
Galactose	75	85	76	76
Arabinose	19	7	17	12
Rhamnose	1	trace	1	2

TABLE V.2

DATA FOR THE GUMS FROM SOME JULIFLORAE SPECIES OF ACACIA

	Acacia auriculiformis	Acacia mangium	Acacia leptostachya	Acacia pubifolia
Ash, %	5.3	5.4	5.8	3.4
Nitrogen,%	0.92	0.98	0.66	1.66
Methoxyl,%	1.68	1.49	2.24	1.20
Specific rotation, degrees	+16	+36	+58	-58
Intrinsic viscosity, ml/g	25	27.7	16.7	25.6
Molecular weight, $\overline{M}_w \times 10^6$	3.0	3.2	1.35	2.44
Equivalent weight	635	545	475	680
Hence uronic acid,%	27.7	32.2	37.0	25.9
Sugar composition after hydrolysis:				
4-O-Methylglucuronic acid	10.1	9.0	13.4	7.2
Glucuronic acid	17.6	23.2	23.6	18.7
Galactose	59	56	54	46
Arabinose	8	10	7	25
Rhamnose	5	2	2	3

number is BRM 4013. According to Mr. Maslin, this is "An undescribed species of unknown affinities, possibly allied to A. dentifera; it has racemes consisting of two flower heads and phyllodes predominantly 1-nerved but with minor secondary, parallel nerves, reminiscent of A. cyclops, whose legumes are, however, very different".

V.3. EXTRACTION AND PURIFICATION OF GUM SAMPLES

Crude gums (2-3g) were dissolved in distilled water (2% solution) over two days.

In the case of Acacia saliciformis a great deal of gum remained undissolved and required mild treatment with sodium borohydride for two days to facilitate almost complete dissolution. The other samples were very soluble in cold water. The solutions were filtered through No. 42 and No.1 filter papers, dialysed against running tap water for two days (four days in the case of the sample treated with borohydride), refiltered, and freeze-dried.

The gums from A. xanthina, A. rostellifera and the new species gave dark brown solutions. The gums from A. saliciformis, A. dictyophleba and A. cyclops gave pale yellow solutions.

V.4. ANALYTICAL RESULTS

Analytical data for the six samples are shown in Table V.3. All the samples were hydrolysed with 0.5M sulphuric acid and the hydrolysates were examined by paper chromatography in solvents (a) and (b).

The gums all contained galactose, arabinose and rhamnose, with galactose the major component. The gums also contained at least two

TABLE V.3

ANALYTICAL DATA FOR PURIFIED GUM POLYSACCHARIDES FROM ACACIA SPECIES OF THE PHYLLODINEAE SERIES

Sample	(a) A. saliciformis	(b) A. xanthina	(c) A. rostellifera	(d) A. dictyophleba	(e) A. cyclops	(f) un-named species
Moisture,%	10.4	3.99	10.2	9.6	9.08	6.66
Ash,%	3.1	3.9	3.0	2.4	3.6	5.8
Nitrogen,%[a]	1.2	0.67	0.18	7.55	0.08	0.23
Hence Protein,%[a]	7.5	4.2	1.12	52.2	0.55	1.47
Methoxyl,%[b]	1.1	1.1	0.75	2.28	0.55	0.38
$[\alpha]_D$ (degrees)[b]	-54	+20°	+90	-32	+26	-32
Intrinsic viscosity, ml/g	39.0	11.0	10.2	15.8	7.4	6.2
Molecular weight, $\bar{M}w \times 10^4$[a]	200	143	95	28	30	51
Equivalent weight	1397	908	687	481	888	564
Hence uronic acid,%[b,c]	13.0	19.0	26	36	20	33
Sugar composition[b] after hydrolysis:-						
4-O-Methylglucuronic acid[d]	6.6	6.6	5	13.7	3.3	2.3
Glucuronic acid	6.4	12.4	21	22.3	16.7	30.7
Galactose	55	52	58	53	68	46
Arabinose	26	29	12	11	5	5
Rhamnose	6	traces	4	traces	7	16

a = corrected for moisture content

b = corrected for moisture content and protein content

c = If all acidity arises from uronic acids

d = If all methoxyl groups located in this acid.

aldobiuronic acids (R_{gal} 0.2 and 0.67 in solvent (b)) which are the most commonly occurring acids in Acacia gums i.e. 6-O-(β-D-gluco-pyranosyl uronic acid)-D-galactose and 4-O-(4-O-methyl-α-D-gluco-pyranosyl uronic acid)-D-galactose.

The gums studied have high molecular weights. The Phyllodinous and bipinnate Australian Acacias studied previously (11, 17, 18) have been of much lower molecular weight than the African species studied (19), but the values for A. saliciformis and A. xanthina are as high as those reported for some Juliflorae species (Table V.2).

In agreement with earlier reports (18) the use of 1% aqueous sodium borohydride (20) does not appear to cause degradation during the dissolution process.

The Phyllodineae species studied show a wide range of intrinsic viscosities, some of them (A. xanthina, A. rostellifera, A. cyclops and the un-named species) give gums of low viscosity, similar to those reported for other species (Table V.1.) A. dictyophleba gum has a higher viscosity, similar to that of the gum from A. leptostachya (Table V.2.). The highest viscosity observed was for A. saliciformis gum (39.0 ml/g) and this must be regarded as the most viscous Acacia gum to be studied so far.

The methoxyl content of these gums is relatively high with the exception of A. cyclops and the un-named species. The value given by A. dictyophleba gum is as high as that recorded previously (21) (for A. giraffae, 2.4%) and is higher than the values for other Juliflorae species (Table V.1).

The nitrogen content for most of these gums lies in the range commonly given by Acacia gums (0.08% to 1.2%), but

A. dictyophleba gum is a notable exception. Its nitrogen content (7.5%) is the highest recorded so far for an Acacia gum. The gum exuded by AZADIRACHTA INDICA (22) contains ca. 5.8% of nitrogen.

The uronic acid content of these gums ranges from 13% to 36%. For many years, A. cyanophylla gum was the most acidic Acacia gum known (23) (uronic acid = 24%). Some Juliflorae species (Table V.2) now have values higher than that figure, however.

The majority of the gums studied in this work have uronic acid values as high as that recorded for A. cyanophylla (23) and the uronic acid content of A. dictyophleba gum (36%) is similar to those reported for some gums of the genus Combretum (17).

Galactose is the major sugar component of the gums studied; the values (50-68%) are comparable to those obtained for other Juliflorae species, but considerably lower than those reported for Phyllodineae species of other sub-groups (15).

The arabinose contents are more variable; the majority of the gums studied in this work contain 5-12% of arabinose but A. saliciformis (26%) and A. xanthina (29%) are exceptions.

Rhamnose, the minor sugar component, has values ranging from 4% to 7%. For A. xanthina gum and A. dictyophleba gum rhamnose could not be detected by paper chromatography. In general the rhamnose values reported here are higher than those obtained for the gums from other phyllodinous Acacias.

The specific rotations obtained range from -54° (A. saliciformis) to +90° (A. rostellifera) and these values extend both the positive and negative ranges of specific rotation published for other gums of the Series Phyllodineae (Tables V.1 and V.2).

The analytical parameters of the gum from the un-named species show that it is not particularly close to A. cyclops gum, and no decision can be reached until analytical data for the gum from A. dentifera can be obtained.

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SECTION VI. A.

AN ANALYTICAL STUDY OF SOME *ACACIA* GUM EXUDATES OF THE SERIES GUMMIFERAE

VI.1. INTRODUCTION

A general introduction to the genus *Acacia* is given in Section V of this thesis.

Bentham's Series 4, Gummiferae, a predominantly African group of *Acacias*, is comprised of 60 species (1), as follows: Sub-series 1, Semibracteatae, 13 spp.; Sub-series 2, Medibracteatae, 39 spp.; Sub-series 3, Basibracteatae, 8 spp.

To date, gums from the following *Acacia* spp. of the Series Gummiferae have been studied in detail: *A. arabica* (2), *A. drepanolobium* (3), *A. nilotica* (4), *A. nubica* (5) and *A. seyal* (6). Table VI.1 shows the data published (2,7) for the gums from these species.

There is analytical evidence that, in terms of fine structure, there are seasonal variations (8,9) in the composition of the gum from any particular *Acacia* species; there are also variations in the composition and properties of the gum exuded by different trees of each particular species. It is therefore important for separate gum specimens from each *Acacia* species to be analysed in order to find the average values for composition of the gum from each species and the variations from these values.

The occurrence of species such as *A. ehrenbergiana*, *A. xanthophloea*, *A. hockii*, which belong to the Gummiferae, are now known to be considerably more widespread in Africa than previously supposed.

An analytical study has been made of the gum exudates from *A. xanthophloea* (3 specimens), *A. ehrenbergiana* (3 specimens), *A. hockii* (2 specimens), *A. sieberana* (1 specimen), and *A. karroo*

TABLE VI.1

Data for some Gummiferae species of *Acacia*

	Acacia drepanolobium	*Acacia nilotica*	*Acacia nubica*	*Acacia seyal*	*Acacia arabica*
Ash,%	2.52	2.48	1.54	2.87	n.d.
Nitrogen,%	1.11	0.02	0.20	0.14	0.07
Methoxyl,%	0.43	0.96	0.05	0.94	0.88
Specific rotation, degrees	+78	+108	+98	+51	+112
Intrinsic viscosity, ml/g	17.8	9.5	9.8	12.1	9.9
Molecular weight, $\bar{M}w \times 10^6$	0.95	2.2	0.87	0.85	2.3
Equivalent weight	1980	1890	3030	1470	1880
Hence uronic acid,%	9	9	7	12	10
Sugar composition after hydrolysis:					
4-O-Methylglucuronic acid	2.5	6	0.5	5.5	6
Glucuronic acid	6.5	3	6.5	6.5	4
Galactose	38	44	33	38	32
Arabinose	52	46	59	46	57
Rhamnose	1	0.4	1	4	0.4

(15 specimens).

VI.2. ORIGIN OF GUM SAMPLES

Gum from *Acacia ehrenbergiana*. Three specimens of the gum exudate from *A. ehrenbergiana* were collected on 16 February 1977 from the outskirts of the airport at Nioro du Sahel, Mali, by Dr. J. Vassal, Botany Dept., University of Toulouse, France.

Two gum specimens from *Acacia hockii* de Willd. (closely related to *A. seyal*), and a specimen from *Acacia sieberana*, D.C. Var. Villosa A. Chev., were collected in Northern Ghana by Dr. M. Jefferies, University of Salford.

Gum (sample 1) from *Acacia xanthophloea*, Bentham No. 336, was collected in Kenya, East Africa, by Dr. J.O. Kokwaro, Botany Dept., University of Nairobi, in August 1976. Gum from *Acacia xanthophloea* (sample 2), sent by Dr. K.D. Gordon-Gray from Pietermaritzburg, South Africa, was obtained from a tree grown from seed collected in Zululand.

Fifteen samples of gum from *Acacia karroo* Hayne were also studied and the data are included in part VI.B.

VI.3. PURIFICATION OF SAMPLES

All samples were dissolved in cold water. After two days the solutions were filtered, dialysed for two days against running tap water, refiltered, and freeze-dried. Good yields of freeze-dried polysaccharides were obtained in all cases.

VI.4. ANALYTICAL RESULTS

Analytical data for the gum exudates from *A. ehrenbergiana*, (3 specimens); *A. xanthophloea* (2 specimens); *A. hockii* (2 speci-

mens); and A. sieberana are shown in Tables VI.2, VI.3, VI.4, and VI.5 respectively.

The analytical parameters for four of these specimens (all members of the Series Gummiferae) are compared in Table VI.6.

All the gum specimens studied were hydrolysed with 0.5M sulphuric acid and the hydrolysates were examined by paper chromatography in solvents (a) and (b), and were shown to contain galactose, arabinose, and rhamnose, with galactose the major component. The gums also contained two aldobiuronic acids with R_{gal} 0.2 and 0.67 in solvent (b); these are the most commonly occurring aldobiuronic acids found in Acacia gums i.e. 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

VI.5. DISCUSSION

The data for the three specimens of gum from A. ehrenbergiana (Table VI.2) shows that although there are a few divergences (e.g. the relatively low negative rotation (-3°) of sample 3 and the relatively low uronic acid content (17.0%) of sample 1,) the three specimens of A. ehrenbergiana gum are analytically very similar.

The gum exudates from A. ehrenbergiana are characterized by their low negative rotation (-6°), low viscosity (8.0 ml/g), intermediate molecular weight (2×10^5), relatively high uronic acid content (20%), a significant rhamnose content (10%) and a galactose: arabinose ratio of 3:1.

There is evidence from the literature (9) that the composition of Acacia gum exudates from different geographical locations may vary. In order to obtain further evidence on this point Bell (10)

TABLE VI.2

Analytical data for three gum specimens from *Acacia ehrenbergiana*

	Specimen		
	1	2	3
Moisture, %	6.0	7.3	12.1
Ash,%	3.1	3.0	3.5
Nitrogen,% [a]	0.09	0.12	0.11
Hence Protein,%[a]	0.56	0.75	0.68
Methoxyl,%[b]	0.84	0.56	0.48
Specific rotation,[b] degrees	-7	-9	-3
Intrinsic viscosity,[a] ml/g	7.2	8.1	8.5
Molecular weight,[a] $\overline{M}w \times 10^4$	27	11	10
Equivalent weight	1063	814	817
Hence uronic acid,% [b,c]	17.0	21.6	21.5
Sugar composition[b] after hydrolysis:-			
4-O-Methylglucuronic acid[d]	5.0	3.4	2.9
Glucuronic acid	12.0	18.2	18.6
Galactose	56	55	51
Arabinose	17	13	16
Rhamnose	10	10	11

[a] Corrected for moisture content.

[b] Corrected for moisture and protein contents.

[c] If all acidity arises from uronic acids.

[d] If all methoxyl groups located in this acid.

TABLE VI.3

Data for two gum specimens from *Acacia xanthophloea*

	Sample 1 (as a nodule)	Sample 1 (as a powder)	Sample 2
Moisture, %	2.7	10.8	11.1
Ash, %	2.4	2.7	5.5
Nitrogen, %[a]	0.14	0.10	0.39
Hence Protein, %[a]	0.87	0.62	2.4
Methoxyl,%[b]	2.4	1.0	2.0
Specific rotation,[b] degrees	+35	+32	+44
Intrinsic Viscosity,[a] ml/g	15	11	24
Molecular weight,[a] $\bar{M}w \times 10^4$	92	86	4
Equivalent weight	1047	1212	1125
Hence uronic acid,%[b,c]	16.8	14.5	15.6
Sugar composition[b] after hydrolysis:-			
4-O-Methylglucuronic acid[d]	14.4	6.2	12.5
Glucuronic acid	2.4	8.3	3.1
Galactose	54	57	61
Arabinose	23	22	16
Rhamnose	6	6	7

a Corrected for moisture content.

b Corrected for moisture and protein contents.

c If all acidity arises from uronic acids.

d If all methoxyl groups located in this acid.

TABLE VI.4

Analytical comparison of the gums from A. hockii and A. seyal

	A. hockii (sub-grade)	A. hockii	A. seyal
Moisture	11.5	4.4	-
Ash,%	3.5	1.3	2.87
Nitrogen,%[a]	0.09	0.23	0.14
Hence Protein,%[a]	0.56	1.44	0.87
Methoxyl,%[b]	0.96	0.61	0.94
Specific rotation,[b] degrees	+41	+91	+51
Intrinsic Viscosity,[a] ml/g	15.3	13.2	12.1
Molecular weight,[a] $\overline{M}w \times 10^4$	128	27.4	85
Equivalent weight	958	1457	1470
Hence uronic acid,%[b,c]	18	12	12
Sugar composition[b] after hydrolysis:-			
4-O-Methylglucuronic acid[d]	5.8	3.7	5.5
Glucuronic acid	12.2	8.3	6.5
Galactose	36	50	38
Arabinose	36	30	46
Rhamnose	10	8	4

a Corrected for moisture content.

b Corrected for moisture and protein contents.

c If all acidity arises from uronic acids.

d If all methoxyl groups located in this acid.

TABLE VI.5

Analytical data for the gum exudate from *A. sieberana*

Moisture	6.04
Ash,%	1.5
Nitrogen,%[a]	0.19
Hence Protein,%[a]	1.2
Methoxyl,%[b]	0.68
Specific rotation,[b] degrees	+103
Intrinsic Viscosity,[a] ml/g	11.8
Molecular weight;[a] $\bar{M}w \times 10^4$	140
Equivalent weight	1232
Hence uronic acid,%[b,c]	14.3
Sugar composition[b] after hydrolysis:-	
4-O-Methylglucuronic acid[d]	4.1
Glucuronic acid	10.2
Galactose	35
Arabinose	46
Rhamnose	4

[a] Corrected for moisture content.

[b] Corrected for moisture and protein contents.

[c] If all acidity arises from uronic acids.

[d] If all methoxyl groups located in this acid.

TABLE VI.6

Data for gums from some Gummiferae species of *Acacia*

	A. ehrenbergiana	*A. hockii*	*A. sieberana*	*A. xanthophloea*
Moisture,%		11.5	6.04	2.7
Ash,%	3.2	3.5	1.5	2.4
Nitrogen,%[a]	0.11	0.09	0.19	0.14
Hence Protein,%[a]	0.66	0.56	1.2	0.87
Methoxyl,%[b]	0.62	0.96	0.68	2.4
Specific rotation[b], degrees	-6°	+41	+103	+35
Intrinsic Viscosity[a], ml/g	7.9	15.3	11.8	15
Molecular weight[a], $\overline{M}w \times 10^4$	16	128	140	92
Equivalent weight	898	958	1232	1047
Hence uronic acid,[b,c]%	20	18	14.3	17
Sugar composition[b] after hydrolysis:-				
4-O-Methylglucuronic acid[d]	3.80	5.8	4.1	14.4
Glucuronic acid	16.2	12.2	10.2	2.6
Galactose	54	36	35	54
Arabinose	16	36	46	23
Rhamnose	10	10	4	6

[a] Corrected for moisture content.

[b] Corrected for moisture and protein contents.

[c] If all acidity arises from uronic acids.

[d] If all methoxyl groups located in this acid.

studied different specimens of the gums from A. cyanophylla and A. pycantha. The composition and properties of an African specimen from Pretoria and of a Western Australian specimen of A. cyanophylla gum did not differ significantly from the values published for a South African sample. A similar conclusion was reached when analytical data for two specimens of A. pycantha gum from New South Wales were compared with the values given in the literature (11) for a South Australian sample.

The analytical parameters (Table VI.3) of A. xanthophloea gum, sample 1 (collected in East Africa) and sample 2 (collected in South Africa), show that these two specimens are analytically very similar. It is also of interest to compare the analytical data for A. xanthophloea, sample 1, before (as nodules) and after it was finely powdered. All the analytical parameters of both samples are very similar, but the methoxyl content is considerably lower in the powdered sample. When the sample was powdered in a cross-beater hammer mill (Glencreston Ltd., London) there was an increase in the temperature of the gum powder that could have led to a loss of methoxyl content.

The analytical data for A. hockii (Table VI.4) show that there are significant differences between the different specimens. It must be emphasised that one specimen of the gum was of poor quality.

There is evidence (12) that each Acacia species produces a characteristic gum exudate; although A. seyal is botanically very close to A. hockii, their gum exudates differ chemically (See Table VI.4).

The analytical data for A. sieberana (Table VI.5) are very similar to those published (13) previously for A. sieberana.

The gums studied have high molecular weights (See Table VI.6), a tendency shown previously by other Gummiferae species (See Table VI.1).

When compared with the other Gummiferae species studied, A. ehrenbergiana gum has a very low viscosity (7.9ml/g). The uronic acid contents of the gums studied range from 14% (A. sieberana) to 20% (A. ehrenbergiana). These values are higher than those observed previously for other Gummiferae species (see Table VI.1). Galactose is the major sugar component of the gums studied and the range (35 - 54%) extends that observed previously for other Gummiferae species (Table VI.1). For rhamnose, however, the values are higher than those reported previously for Gummiferae species (Table VI.1). The rhamnose contents of the gums from A. ehrenbergiana and A. hockii (10%) are comparable with those published for species of Bentham's Series 5, Vulgares (7) which are characterized by the presence of significant proportions of rhamnose.

The specific rotations of the gums studied have high positive values but A. ehrenbergiana gum (-6°) is an exception.

SECTION VI.B.

VARIATIONS IN THE COMPOSITION AND PROPERTIES OF THE GUM EXUDED BY *ACACIA KARROO* HAYNE

VI.B.1. INTRODUCTION

According to Ross (14, 15), *Acacia karroo* Hayne is the most widespread *Acacia* in southern Africa, numerically well-represented throughout most of its range and exceedingly variable, especially in Natal (16), where at least six variants, ranging from large trees to small fire-resistant shrubs, are recognized. Although the most extreme of these variants differ very considerably, Ross found that they all tended to grade, without clearly definable limits, into a central gene pool so that none of them should be accorded infra-specific rank. The great variability of *A. karroo* is the result of its ability to exploit many diverse habitats and adapt to a wide range of environmental conditions.

The only analytical data available previously for *A. karroo* gum were for a specimen collected near Alice, Eastern Cape Province (17). In 1977, specimens of gum exuded by trees attacked by wood-boring beetles were collected during a study of the ecology and feeding habits of bush-babies (Galagidae). Information was required concerning the extent to which this gum can be regarded as typical of *A. karroo*, and whether it could plausibly be regarded as a source of the nutrients (carbohydrate, protein and minerals) essential for the bush-babies (18), which appear to be able, when other foodstuffs are scarce, to exist on a diet of gum and arthropods. Comparative analysis of these, and of a variety of other specimens of *A. karroo* gum collected in widely separated regions of Africa, have therefore been carried out.

VI.B.2. ORIGIN OF GUM SPECIMENS FROM A. KARROO

Gum specimens 1-5 (Table 1) were collected on 14, 15, 17, 25 and 30 September 1976 from A. karroo trees growing near Naboomspruit, Northern Transvaal (28°47'E, 24°35'S); these trees were under close observation (Site 16) during a field study of the ecology and feeding-habits of bush-babies.

Specimens 6-10 (Table 1) were collected at Kumkuas, District Grootfontein, Namibia, as follows: specimens 6 and 7 by Mr. H.D. von Alvensleben on 29 August and 8 September 1975, respectively; specimens 8-10 by Mr. W. Giess on 28 August 1975. Specimens 6 and 8 were clear, good quality gum exudates; specimens 7, 9 and 10 were of frass (mixtures of gum and wood debris) from beetle-borer activity.

Specimens 11-13 were collected by officers of the Botanical Research Institute, Pretoria; specimen 11 at Kimberley on 2 September 1963 and specimens 12 and 13, respectively, from the Research Institute Garden, Pretoria, on 18 and 24 April 1964. Specimens 14 and 15 were collected in December 1975 by Professor K.D. Gordon-Gray from two trees, 500m apart, belonging to the dry river valley (typical) race of A. karroo growing in the Tugela Valley, near Tugela Ferry (30°30'E, 28°45'S). The gum was collected from the ground, directly beneath partly severed branches caused by natural damage.

VI.B.3. RESULTS AND DISCUSSION

The analytical data for the 15 gum specimens are given in Table VI.B.1, in which the final column shows the average

Table VI.B.1 Analytical data for gum specimens from *Acacia karroo* collected in Africa

	Samples															
	Northern Transvaal (collected by London Zoo, 1976)					Namibia (collected by W. Giess, 1975)					Kimberley, Pretoria (collected by Botanical Research Institute, 1963)			Tugela Valley (collected by Gordon-Gray, 1975)		Average all samples
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Moisture (%)	3.2	8.4	7.1	11.1	9.4	6.8	3.2	5.8	2.3	2.5	11.4	11.1	9.7	1.8	6.7	6.7
Ash (%)	2.3	3.0	3.2	3.3	3.6	n.d.	2.5	n.d.	2.8	2.7	3.8	3.4	3.4	4.2	3.2	3.2
Nitrogen (%)	0.11	0.13	0.13	0.17	0.11	0.09	0.16	0.15	0.12	0.12	0.15	0.19	0.24	0.17	0.17	0.15
Hence protein (%) (N × 6.25)	0.69	0.81	0.81	1.06	0.69	0.56	1.0	0.94	0.75	0.75	0.94	1.18	1.50	1.06	1.06	0.92
Methoxyl (%)	0.40	0.34	0.32	0.30	0.44	0.80	0.70	0.65	0.44	0.48	0.40	0.45	0.42	0.49	0.43	0.47
Specific rotation, $[\alpha]_D$, degrees	+59	+56	+46	+52	+42	+49	+49	+58	+50	+67	+49	+48	+48	+42	+38	+53
Intrinsic viscosity, $[\chi]$, ml g^{-1}	19.2	20.0	22.6	20.4	20.0	10.5	11.2	12.8	15.9	3.2	18.7	18.0	28.8	17.7	17.0	17.1
Molecular weight, $\bar{M}w \times 10^5$	48	41.5	44	38.7	39.9	1.9	3.3	1.5	2.7	4.6	3.8	2.9	11.5	21.8	13.7	18.6
Equivalent weight	1322	1318	1309	1270	1330	1548	1707	1483	1212	1180	969	986	1067	988	1103	1253
Hence uronic anhydride (%)	13.3	13.3	13.4	13.8	13.2	11.4	10.3	11.9	14.5	14.9	18.1	17.8	16.5	17.8	16.0	14.4
Sugar composition after hydrolysis																
4–O–methylglucuronic acid	2.4	2.0	1.9	1.8	2.6	4.8	4.2	3.9	2.6	2.9	2.4	2.7	2.5	2.9	2.6	2.8
Glucuronic acid	10.9	11.3	11.5	12.0	10.6	6.6	6.1	8.0	12.0	12.0	15.7	15.1	14.0	14.9	13.4	11.6
Galactose	42	48	47	51	50	47	47	52	54	55	49	54	58	53	50	50
Arabinose	40	33	33	28	31	32	34	27	26	26	25	22	20	22	28	28
Rhamnose	5	6	6	7	6	10	8	9	5	4	8	6	6	7	6	7

for each of the analytical parameters studied. The limited analytical data published originally for A. karroo gum (17) viz. $(\alpha)D = +54^{\circ}$, equivalent weight 1660; glucuronic acid, 12%; D-galactose, 50%; L-arabinose, 36%; L-rhamnose, 2%, show reasonable agreement with these average values.

The only Acacia species for which comparable number of separate gum specimens have been analysed are Acacia seyal Delile (19), A. nilotica (L.) Willd. ex Delile (20), A. laeta R. Br. (21) and A. senegal (L.) Willd. (22). The ranges of values recorded for the analytical parameters of each of these gum species are consistently narrower than those now reported for A. karroo, even in the very variable A. senegal. The data for A. karroo show an interesting combination of similarities and differences. Thus specimens 14 and 15 are very similar, as might be expected for two trees growing closely together; yet specimens 12 and 13, from trees growing in the same garden, are closely alike in composition but surprisingly different in terms of their molecular weight and intrinsic viscosity. The Namibian specimens, 6-10, consistently show the lowest molecular weights and intrinsic viscosities of all the specimens studied; they also show the greatest variations in composition, e.g., rhamnose 4-10%; uronic acid 10.3%-14.9%. Specimens 6-10 are a particularly interesting group, as three of them were samples of gum frass resulting from attacks by borer-beetles. In the only previous studies (22) of such gum frass, exuded by A. senegal trees infested with longhorn (Cerambycidae) or jewel (Buprestidae) beetles, the composition and structure of the gum extracted

from the frass was found to be closely similar to that of normal, tapped, gum exudate.

Specimens 1-5 form a homogeneous group: only specimen 1 shows an unusual feature, i.e., a ratio for galactose to arabinose of 42 : 40. The most interesting feature of all the specimens studied involves the very high molecular weight given by specimens 1-5 in response to attacks by wood-boring insects, tentatively identified (Beardor & Martin, personal communication) as Coccid moth larvae, Buprestid and Cerambycid beetles. The A. karroo trees concerned were identified from photographs as typical A. karroo with dark, rough, fissured bark, sessile bright yellow flowers, and long white stipules, of variable length, beneath the nodes. These trees grow on ground that is subject to occasional seasonal flooding to a depth of several feet; attempts to induce the trees to yield gum by gouging holes of different diameters and different depths did not lead to the flow of as copious amounts of gum as that resulting from beetle attack (Beardor & Martin, personal communication). Ross (14) pointed out that, of all the indigenous Acacia species, A. karroo is the most prone to severe attacks by the wattle bagworm, Kotchalia junodii Heyl. which frequently kills fairly large trees.

At the site of the ecological study, trees of Acacia karroo, A. tortilis (Forsk.) Hayne subsp. heteracantha (Burch) Brenan and A. nilotica were all abundant, but the susceptibility of these species to attack by wood-boring beetles differed greatly: trees of A. karroo were most strongly attacked; trees of A. nilotica were attacked to a very small extent, with trees of A. tortilis

subsp. heteracantha in an intermediate position. Accordingly, the major source of gum available to the bush-babies was that from A. karroo.

From the analytical data now available for the Acacia gums involved, the gum from A. nilotica (20) is the least proteinaceous. Subspecies heteracantha, which gives the most proteinaceous gum of the A. tortilis complex (23) and is of similarly high intrinsic viscosity and molecular weight to that of A. karroo specimens 1-5 has, nevertheless, a very different composition (galactose:arabinose ≃ 24 : 62). A complex biological factor involving the abundance of the gum supply rather than the proportions of carbohydrate and/or protein available in the gum from a particular species may therefore be involved. Although the regeneration of A. tortilis subsp. spirocarpa (Hochst. ex A. Rich.) Brenan is threatened because its seeds are particularly susceptible to attack by Bruchidus beetles, seeds of the closely related A. tortilis subsp. heteracantha are not so vulnerable, and its timber is not as vulnerable as that of A. karroo. Other similar preferences by predators are known: yellow baboons (Papio cynocephalus L.) greatly prefer the gum exudate from A. xanthophloea Bentham to that from A. tortilis as a foodstuff (24). Studies of A. xanthophloea gum are reported in Section VII of this thesis.

It will be of interest to study the variability of A. karroo gum more comprehensively if specimens of gum from the other white-bark, short-stipuled, and shrubby variants described by Ross (14) can be obtained for study from collectors in as widely differing geographical locations as possible.

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SECTION VII

STRUCTURAL STUDY

OF THE GUM FROM *ACACIA XANTHOPHLOEA*

VII. 1. INTRODUCTION

The gum exudates from trees of the genus Acacia are probably the most widely studied by chemists active in the field of gum research. The most detailed studies have been made on gums from Acacia species in Series 4 (Gummiferae) and and Series 5 (Vulgares) of Bentham's division of the genus. The gums from species in Series 4 appear to be more complex structurally than those from Series 5. Comparisons of the data available for these gums have been reported (1).

This section reports a study of some of the structural features of the gum from A. xanthophloea and compares these with those of other gums e.g. from A. senegal (2), A. seyal (3), A. drepanolobium (4), A. arabica (5), for which the structures have been well characterized.

VII. 2. ORIGIN AND PURIFICATION OF SAMPLE

The origin of A. xanthophloea gum, sample 1, was described in Section VI of this thesis.

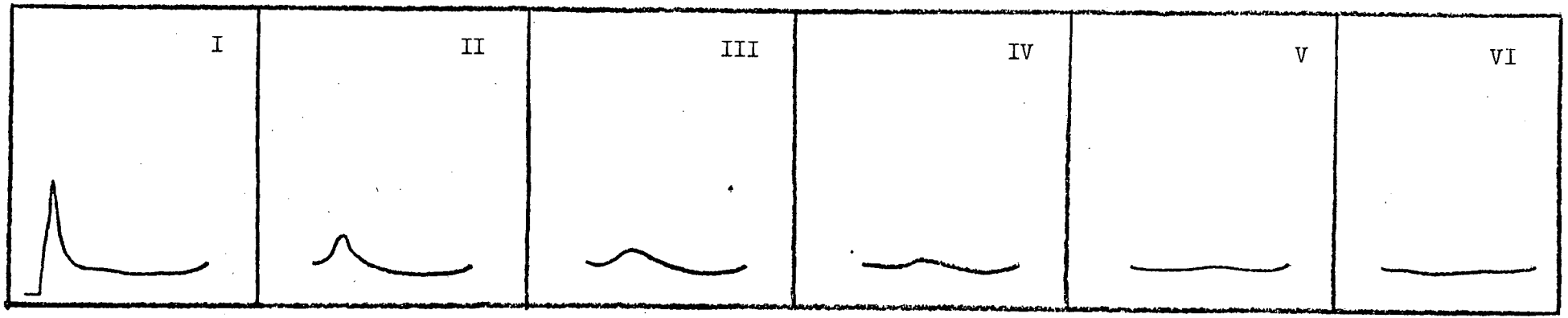
Crude gum (160g), finely powdered, was dissolved in water (3.5 l). The gum solution was filtered and dialysed against running tap-water for two days. The polysaccharide (128g, yield 80%) was isolated as the freeze dried product.

VII. 3. ANALYTICAL DATA

The analytical data obtained for this sample were given in Section VI.A.

Ultracentrifugation of the sample gave one symetrical peak, (Fig. VII.1).

Fig. VII.1 Sequence of ultracentrifuge (44,000 r.p.m.) photographs (Schlieren) at 16 min. intervals



VII. 4. IDENTIFICATION OF NEUTRAL SUGARS

Acacia xanthophloea gum (5g) was hydrolysed with 0.5M-sulphuric acid (250ml) for 8 hours on a boiling water bath. The cooled solution was neutralised with barium carbonate, filtered, deionised with Amberlite IR - 120(H) resin and concentrated to a syrup. The syrup was applied to a column (41 x 26cm) of Duolite A - 4 resin in the formate form. Elution with distilled water (700 ml) yielded the neutral sugars present in the hydrolysate. After concentration to a syrup, chromatography with solvents (a) and (b) showed the presence of galactose (main component), arabinose and rhamnose.

VII. 5. IDENTIFICATION OF THE ALDOBIURONIC ACIDS

After elution of the neutral sugars, elution with 5% formic acid (500ml) yielded the acidic fraction of the hydrolysate, which was concentrated to a syrup (after formic acid was removed by repeated addition of water followed by concentration to a syrup), and chromatography was carried out in solvent (a). Trace amounts of galactose was observed along with two main fractions, i.e. fraction A (brown spot (aniline oxalate) with R_{gal} 0.26) and fraction B (pink spot (aniline oxalate) with R_{gal} 0.62). The chromatogram also showed the presence of traces of a fraction (pink spot (aniline oxalate) with R_{gal} 2.32,) which correspond to 4 - O - methyl - D - glucopyranosyluronic acid.

Chromatography was carried out on Whatman 3 MM papers in solvent (a); side strips were cut and sprayed to locate the sugars and the two major acidic fractions were eluted as in sugar ratio

determinations. After elution each fraction was concentrated to a syrup, and the presence of only one component was checked by chromatography in solvent (a). Then in each case the syrup was transferred to a dry, tared 50-ml round-bottom flask; after being taken just to dryness, the weight of aldobiuronic acid was found. Distilled water (2 ml) was added to dissolve the acid and its specific rotation was determined.

Fraction A had R_{gal} 0.21 in solvent (a). After elution, the value of $[\alpha]_D$ was $+2^{\circ}$. Hydrolysis in 1M - sulphuric acid of a portion of this acid followed by chromatography in solvents (a) and (c) showed the presence of galactose and glucuronic acid only. The value obtained for $[\alpha]_D$ suggests a β - linkage. The syrup (9mg) was methylated by the Kuhn method. Methanolysis followed by g.l.c. examination of the methyl glycosides formed indicated the presence of 2,3,4 - tri - O - methyl - D - glucuronic acid (T 2.26, 3.01), 2,3,5 - tri - O - methyl - D - galactose (T4.12, 4.74) and 2,3,4 - tri - O - methyl - D - galactose (T 6.70). This indicates that the aldobiuronic acid is 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose.

Fraction B had R_{gal} 0.62 in solvent (a), with $[\alpha]_D$ + 91°. Hydrolysis, followed by chromatography in solvents (a) and (c) indicated the presence of galactose and 4 - O - methyl - D - glucopyranosyluronic acid. According to hydrolysis studies and physical properties fraction B is 4 - O (4 - O - methyl - α - D - glucopyranosyluronic acid) - D - galactose, which is one of the aldobiuronic acids found most frequently in Acacia gums.

A portion of the gum (100 mg) was hydrolysed with 1M - sulphuric acid and examined by chromatography in solvent (c). Glucopyranosyluronic acid and its 4 - O - methyl analogue were the only uronic acids detected, in agreement with the evidence obtained above from the examination of the aldobiuronic acids.

VII. 6. PARTIAL HYDROLYSIS OF A. XANTHOPHLOEA GUM

Hydrolysis of the gum (100 mg) with 0.25M sulphuric acid (10ml) for 1 hour on a boiling water bath, followed by neutralisation, deionisation, concentration to a syrup and chromatography in solvent (b) showed the presence of a suspected trisaccharide with R_{gal} 0.12, and three disaccharides viz. 6 - O - β - D - galactopyranosyl - D - galactose (major component; R_{gal} 0.35 (brown spot)); 3 - O - β - L - arabinopyranosyl - L arabinose (R_{gal} 0.80 (pink spot)); and a component with R_{gal} 0.70 (pink spot), which was eluted from Whatman 3 MM paper after separation for 60 hours in solvent (b). The resulting syrup (25.9mg) had $[\alpha]_D$ + 50°. Hydrolysis with 0.5M sulphuric acid, followed by chromatography in solvents (a) and (b), showed the presence of galactose and arabinose only, indicating that the biose is 3 - O - β - D - galactopyranosyl - L - arabinose (R_{gal} = 0.70, $[\alpha]_D$ = 50°).

VII. 7. METHYLATION OF ACACIA XANTHOPHLOEA GUM

The gum (442mg) was methylated to give a product (200mg) with $[\alpha]_D$ + 8.3° (C 1.21 in chloroform) and OMe = 45.9%. Methanolysis of a portion of the methylated product followed by g.l.c. examination of the methyl glycosides gave the results shown in Table VII. 1.

TABLE VII.1

METHYL GLYCOSIDES FROM METHYLATED ACACIA XANTHOPHLOEA

Relative retention time (T) of methyl glycosides	R_G after hydrolysis solvent (C)	O-methyl sugars	Relative proportions*
0.32	-	unknown sugar	(4)
0.37	-	unknown sugar	(10)
0.47	1.01	2,3,4 -tri-O-methyl-L-rhamnose	(7)
0.65; 0.71	0.95	2,3,5-tri-O-methyl-L-arabinose	(7)
1.05,	0.79	2,3,4-tri-O-methyl-L-arabinose	(2)
1.05; 2.35		3,5-di-O-methyl-L-arabinose	(4)
1.32; 2.14	0.79	2,5-di-O-methyl-L-arabinose	(40)
1.85		3,4-di-O-methyl-L-arabinose	(7)
1.67	0.87	2,3,4,6-tetra-O-methyl-D-galactose	(6)
2.35		2,3,6-tri-O-methyl-D-galactose	(1)
4.34	0.65	2,4,6-tri-O-methyl-D-galactose	trace
5.93	0.65	2,3,4-tri-O-methyl-D-galactose	trace
2.35; 2.76	-	2,3,4-tri-O-methyl-D-glucuronic acid**	(12)
	0.41	2,4-di-O-methyl-D-galactose	
	0.26	2-O-methyl-D-galactose	trace

* The relative proportions of O-methyl-sugars in this and the following tables are obtained by comparing the areas under individual peaks with the total area of the peaks obtained on the chromatogram.

** as methyl ester glycoside

Hydrolysis of the methyl glycosides, followed by paper chromatography in solvent (d) showed the presence of 2 - O - methyl - D - galactose and 2,4 - di - O - methyl - D - galactose in addition to the methyl glycosides shown. The main component identified was 2, 5 - di - O - methyl - L - arabinose with 2,3,4 - tri - O - methyl - D - glucuronic acid, 3,4 - di - O - methyl - L - arabinose, 2,3,5 - tri - O methyl - L - arabinose, 2,3,4 - tri - O - methyl - L - rhamnose and 2,3,4,6 - tetra - O - methyl - D - galactose also present in large amounts.

VII. 8. PREPARATION OF DEGRADED GUM A

Purified gum (6.0g, dry weight) was hydrolysed with 0.01N sulphuric acid (325.5ml) on a boiling water bath for 96 hours. The brown solution was neutralised with barium carbonate, filtered, concentrated, dialysed against distilled water (2l) for 24 hours and then against running tap water for a further 48 hours, and freeze-dried to give degraded gum A (2.3g; yield 38.3%).

The 24 hour dialysate, concentrated to a syrup and chromatographed in solvents (a) and (b) gave large amounts of arabinose, galactose, and rhamnose, together with three other components. The first of these had R_{gal} 0.14 (solvent (a); brown spot) and R_{gal} 0.11 (solvent (b)) and was a triose (see study of degraded gum A below); the second had R_{gal} 0.27 (solvent (b); brown spot) and was chromatographically identical to 6 - O - β - D - glucopyranosyl - D - galactose; the third had R_{gal} 0.75 (solvent (b), pink spot) and was chromatographically identical to 3 - O - β - D - arabinopyranosyl - L - arabinose.

TABLE VII.2

ANALYTICAL DATA FOR DEGRADED GUM A

Moisture,%	8.6
Ash,%[a]	3.3
Nitrogen, %[a]	0.19
Hence Protein, %[a]	1.2
Methoxyl,%[b]	1.0
Optical rotation,[b] degrees	+18
Intrinsic Viscosity,[a] ml/g	n.d.
Molecular weight,[a] $\overline{M}w \times 10^5$	3.2
Equivalent weight	946
Hence Uronic acid,%[b,c]	19
Sugar composition[b] after hydrolysis:-	
4 - O - methylglucuronic acid[d]	6
Glucuronic acid	13
Galactose	69
Arabinose	4
Rhamnose	8

[a] Corrected for moisture content.

[b] Corrected for moisture and protein contents.

[c] If all acidity arises from uronic acid.

[d] If all methoxyl groups are located in this acid.

n.d. not done

VII. 9. EXAMINATION OF DEGRADED GUM A

Analytical data for degraded gum A are given in Table VII.2. The proportion of uronic acid in degraded gum A is greater than that in the whole gum because 0.01N acid hydrolysis does not break uronosyl linkages.

Hydrolysis of degraded gum A with 0.5M sulphuric acid, followed by chromatography in solvents (a) and (b) gave galactose (major component), arabinose, rhamnose and the same trisaccharide (R_{gal}0.14) observed in the dialysate from the preparation of degraded gum A. This component at R_{gal}0.14 was hydrolysed with 0.5M sulphuric acid and the hydrolysate was shown by chromatography (solvents (a) and (b)), to contain galactose only. Partial acid hydrolysis (with 0.25M acid) gave galactose and 6 - O - β - D - galactopyranosyl - D - galactose. These results indicate that this component was probably the β - 1,6 - linked galacto triose.

Partial acid hydrolysis with 0.25M sulphuric acid for 1 hour, followed by chromatography in solvents (a) and (b) revealed only one major component with R_{gal} 0.27 (solvent (a)) and R_{gal}0.30 (solvent (b)), which was chromatographically identical to 6 - O - β - D - galactopyranosyl - D - galactose. A minor component detected at R_{gal}0.53 was chromatographically identical to 3 - O- β - D - galactopyranosyl - D - galactose. Degraded gum A (270mg) was methylated to give a product (118mg). Found; $[\alpha]_D$ - 29.0°(C 0.96, chloroform); OMe 46.5% A portion of this product was methanolysed and the mixture of methyl glycosides was examined by g.l.c. The methanolysate

TABLE VII.3

METHYL GLYCOSIDES FROM METHYLATED DEGRADED GUM A

Relative retention time (T) of methyl glycosides	R_G after hydrolysis solvent (C)	O-methyl sugars	Relative proportions
0.31	-	unknown sugar	trace
0.37	-	unknown sugar	(3)
0.46	1.01	2,3,4-tri-O-methyl-L-rhamnose	(1)
0.64	0.95	2,3,5-tri-O-methyl-L-arabinose	(0.5)
1.11; 2.07		3,5-di-O-methyl-L-arabinose	(25)
1.30; 2.30		2,5-di-O-methyl-L-arabinose	(2)
1.61	0.87	2,3,4,6-tetra-O-methyl-D-galactose	(1)
2.55;	0.71	2,3,6-tri-O-methyl-D-galactose	(4)
2.81; 4.11	0.71	2,4,6-tri-O-methyl-D-galactose	(10)
4.70	0.71	2,3,4-tri-O-methyl-D-galactose	(27)
11.0; 12.1	0.43	2,4-di-O-methyl-D-galactose	(16)
2.30; 2.81	-	2,3,4-tri-O-methyl-D-glucuronic acid*	(10)

was hydrolysed and the resulting syrup examined by paper chromatography in solvent (d). The O-methyl sugars identified are shown in Table VII.3. The major components were 2,3,4 - tri - O - methyl - D - galactose and 3,5 - di - O - methyl - L - arabinose with substantial amounts of 2,4,6 - tri - O - methyl - D - galactose, 2,4 - di - O - methyl - D - galactose, and 2,3,4 - tri - O - methyl - D - glucuronic acid.

VII.10. PREPARATION OF DEGRADED GUM B

Preliminary small scale experiments indicated that 0.25M sodium metaperiodate solution and an oxidation time of 72 hours were required for the complete oxidation of degraded gum A.

Degraded gum A (1.7g) was dissolved in water (50ml) and 0.5M sodium metaperiodate (50ml) was added. After oxidation had been carried out for 72 hours in darkness at room temperature, 23.4 m moles periodate/g polysaccharide had been reduced and 4.56 m moles formic acid/g polysaccharide released. The reaction was stopped by the addition of ethylene glycol (2ml) and the solution was dialysed for 2 days. After sodium borohydride (1.0g) was added, the solution was left for 30 hours, dialysed for 2 days, made 0.5M with respect to sulphuric acid, hydrolysed for 48 hours at room temperature, then dialysed for 2 days. Degraded gum B was isolated as the freeze-dried product (0.1g, yield 5.3%). The preparation of degraded gum B was repeated and the yield obtained was similar.

VII.11. EXAMINATION OF DEGRADED GUM B

Degraded gum B was hydrolysed with 0.5M - and 1M - sulphuric acid. Chromatography indicated that galactose was

the only sugar present. No trace of aldobiuronic acid was present.

Degraded gum B had $[\alpha]_D$ - 5.5° and the protein content (from N x 6.25) was 5.2%.

Degraded gum B was not obtained in sufficient amount to allow its examination by methylation analysis but it was studied by n.m.r. spectroscopy (See Sections VIII and IX).

VII.12. PREPARATION OF POLYSACCHARIDE I FROM WHOLE GUM

Preliminary, small scale experiments indicated that 0.125M sodium metaperiodate solution and an oxidation time of 96 hours were required to oxidise Acacia xanthophloea gum.

Purified gum (42.3g) was dissolved in water (1058ml) and 0.25M sodium metaperiodate solution (1058ml) was added. Oxidation was carried out in darkness at room temperature and the oxidation was followed by measuring the release of formic acid with time. Aliquots (1ml) of the solution were titrated with 0.1010 N sodium hydroxide with methyl red as indicator (Figure VII.2). After 96 hours, 10.6 m moles periodate/g polysaccharide had been reduced and 1.5 m moles formic acid/g polysaccharide had been released. The reaction was stopped by the addition of ethylene glycol (25ml) and the solution was dialysed for 2 days. Sodium borohydride (12.7g) was added and the mixture was kept at room temperature for 30 hours, dialysed for 2 days, made 0.5M with respect to sulphuric acid by the addition of 2M acid, and the derived polyalcohol was hydrolysed at room temperature for 48 hours. A portion of this solution (ca. 10% of the total solution

Fig. VII.2.

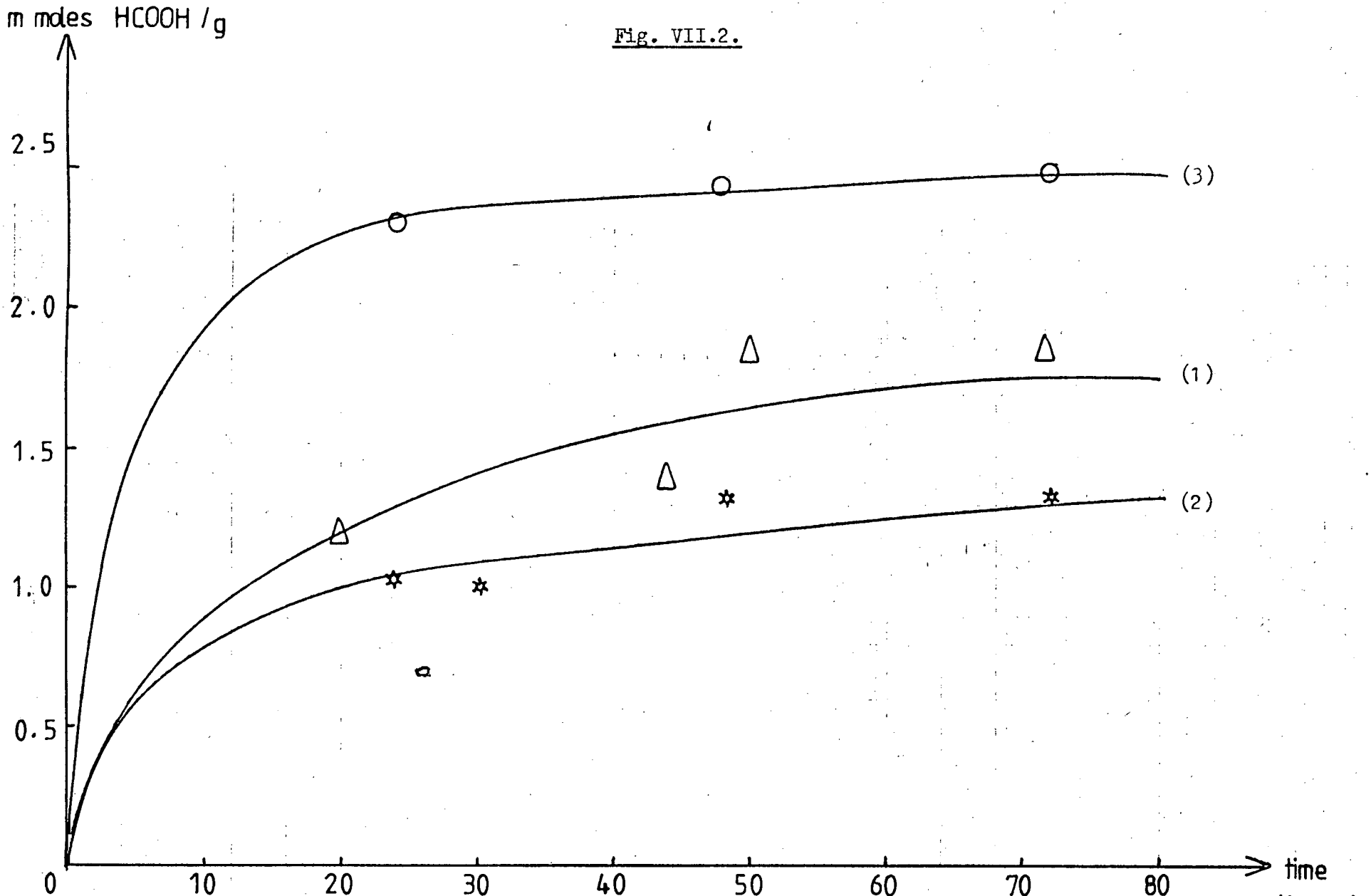


Fig. VII.2. Formic acid released (m moles/g) with time (hours) on periodate oxidation of:
(1) Acacia xanthophloea gum (2) Polysaccharide I (3) Polysaccharide II

volume) was neutralised with barium carbonate, filtered, dialysed against distilled water (4l) for 24 hours and against running tap water for 3 days. Polysaccharide I was isolated as the freeze-dried product (14.0; yield 33%).

The dialysate obtained after the hydrolysis stage in the preparation was concentrated, deionised with Amberlite IR - 120 resin, and concentrated to a syrup. Paper chromatography in solvent (d) indicated the presence of large amounts of arabinose (R_f0.15; pink spot), and glycerol (R_f0.34; brown spot). Paper chromatography in solvent (b) showed the presence of arabinose and 3 - O - β - L - arabinofuranosyl - L - arabinose (R_{gal} 1.20; pink spot).

VII.13. EXAMINATION OF POLYSACCHARIDE I

Analytical data for polysaccharide I are given in Table VII.4. Hydrolysis (0.5M) followed by chromatography in solvents (a) and (b) showed galactose as the major component. Partial acid hydrolysis of polysaccharide I with 0.25M sulphuric acid followed by chromatography in solvents (a) and (b) revealed the presence of a component (possibly a trisaccharide) at R_{gal} 0.06 (solvent (a) and R_{gal}0.12 (solvent (b)) and three disaccharides. The first of these had R_{gal} 0.25, brown spot, (solvent (a), R_{gal}0.31 (solvent (b)), and was 6 - O - β - D - galactopyranosyl - D - galactose; the second had R_{gal}0.56 (solvent (a), R_{gal}0.64 (solvent (b)) and was 3 - O - β - D - galactopyranosyl - D - galactose, and the third, with R_{gal}0.80 (pink spot), solvent (a), was 3 - O - β - L - arabinopyranosyl

TABLE VII.4

ANALYTICAL DATA FOR POLYSACCHARIDE I

Moisture,%	0.63
Ash,%[a]	1.7
Nitrogen,%[a]	0.40
Hence Protein,%[a]	2.5
Methoxyl content,%[b]	0.12
Optical rotation,[b] degrees	-18
Intrinsic Viscosity,[a] ml/g	12.7
Molecular weight,[a] $\bar{M}w \times 10^5$	2.1
Equivalent weight	4843
Hence Uronic acid,%[b,c]	4
Sugar composition[b] after hydrolysis:-	
4 - O - methylglucuronic acid[d]	0.7
Glucuronic acid	3.3
Galactose	70
Arabinose	26
Rhamnose	trace

[a] Corrected for moisture content.

[b] Corrected for moisture and protein contents.

[c] If all acidity arises from uronic acid.

[d] If all methoxyl groups are located in this acid.

- L - arabinose.

Polysaccharide I (295 mg) was methylated to give a product (207mg: yield 70.3%). Found:- $[\alpha]_D$ - 54.2° (C 1.67, chloroform); OMe 39%. A portion of this product was methanolysed and the mixture of methylglycosides was examined by g.l.c. The methanolysate was then hydrolysed (0.5M) and the resulting syrup was examined by paper chromatography in solvent (d). The O - methyl sugars identified are shown in Table VII.5.

VII.14. PREPARATION BY SEQUENTIAL SMITH-DEGRADATION OF POLYSACCHARIDES II-III.

Preliminary, small scale experiments indicated that 0.125M sodium metaperiodate solution and an oxidation time of 76 hours were required for polysaccharides I and II.

The following weights of polysaccharides, in a sequence of Smith-degradations, were oxidised with periodate (0.125M), reduced with borohydride, hydrolysed with sulphuric acid (0.5M) and the corresponding products recovered, all as already described for polysaccharide I. Polysaccharide I (11.0g) gave polysaccharide II (1.7g). To obtain more material for examination, and to check on the low yields obtained, the preparation of polysaccharides I and II was repeated; the yields and properties of the products were very similar to those recorded above. Polysaccharide II (1.7g) gave polysaccharide III (88.7mg). All weights are corrected for moisture. The analytical data for each product are given in Tables VII.6 and VII.7., and the formic acid released with time for Polysaccharides I, II and degraded gum A is shown in Figure VII. 2. (p.83).

TABLE VII.5

METHYL GLYCOSIDES FROM METHYLATED POLYSACCHARIDE I

Relative retention time (T) of methyl glycosides	R_G after hydrolysis solvent (C)	O-methyl sugars	Relative proportions
0.37	-	unknown sugar	(18)
0.48	1.01	2,3,4-tri-O-methyl-L- rhamnose	(8)
0.63	0.95	2,3,5-tri-O-methyl-L-arabinose	trace
1.16; 2.14		3,5-di-O-methyl-L-arabinose	(21)
1.35	0.79	2,5-di-O-methyl-L-arabinose	(7)
1.68	0.87	2,3,4,6-tetra-O-methyl-D-galactose	(4)
2.69; 2.98	0.71	2,4,6-tri-O-methyl-D-galactose	(21)
5.08	0.71	2,3,4-tri-O-methyl-D-galactose	(14)
13.43	0.46	2,4-di-O-methyl-D-galactose	(trace)
2.14; 2.69	-	2,3,4-tri-O-methyl-D-glucuronic acid	(7)
	0.29	2-O-methyl-D-galactose	(trace)

TABLE VII.6

SUGAR RATIOS FOR *ACACIA XANTHOPHLOEA* AND ITS DEGRADATION PRODUCTS

Polysaccharide	Constituent sugars				
	gal.	ara.	rha.	glu. acid	4-O-methylglu. acid
A. xanthophloea gum	57	22	6	8.8	6.2
Degraded gum A	69	4	8	13.0	6.0
Degraded gum B	100	-	-	-	-
Polysaccharide I	70	26	trace	3.3	0.7
Polysaccharide II	90	10	-	-	-
Polysaccharide III	100	-	-	-	-

TABLE VII. 7

RELATIVE PROPORTIONS OF O-METHYL SUGARS

IN POLYSACCHARIDES I AND II

O-methyl sugar	Polysaccharide I	Polysaccharide II
2,3,4-tri-O-methyl-L-rhamnose	9.0	-
2,3,5-tri-O-methyl-L-arabinose	trace	-
3,5-di-O-methyl-L-arabinose	23	29
2,5-di-O-methyl-L-arabinose	7	trace
2,3,4,6-tetra-O-methyl-D-galactose	4	trace
2,4,6-tri-O-methyl-D-galactose	24	62
2,3,4-tri-O-methyl-D-galactose	15	9
2,4-di-O-methyl-D-galactose	trace	trace
2,3,4-tri-O-methyl-D-glucuronic acid	7	-

Partial acid hydrolysis (0.25M sulphuric acid for 1 hour at 100°C) of polysaccharides II and III, followed by chromatography in solvent (b) showed the presence of 6 - O - β - D - galactopyranosyl - D - galactose (R_{gal} 0.31) and 3 - O - β - D - galactopyranosyl - D - galactose (R_{gal} 0.53). An oligosaccharide at R_{gal} 0.14, gave only galactose on hydrolysis.

Hydrolysis with sulphuric acid (1M), followed by chromatography in solvents (a) and (c) showed the presence of galactose and arabinose. Uronic acids and rhamnose were not detected. The ratio of galactose:arabinose in Polysaccharide II was 9 : 1.

Polysaccharide II was methylated in the normal way and a portion of the product was methanolysed and examined by g.l.c. The methyl glycosides found are shown in Table VII.7.

Table VII.8 shows analytical data for A. xanthophloea gum and its degradation products.

Polysaccharide III, obtained in insufficient amount to allow its examination by methylation analysis, was examined by n.m.r. spectroscopy (See Sections VIII and IX).

VII.15. DISCUSSION

Purified gum from Acacia xanthophloea was examined (by courtesy of Mr. Hart, Botany Dept., Edinburgh University) by ultracentrifugation and this experiment indicated clearly that a one component system is involved. (See Fig. VII.1).

Complete hydrolysis of the gum with 0.5M acid showed that it consisted of galactose (57%), arabinose (22%), rhamnose (6%) and uronic acid (15%). Partial acid hydrolysis (with 0.25M acid) showed the presence of 6 - O - β - D - galactopyranosyl - D - galactose (major component) and 3 - O - β - L - arabinopyranosyl - L - arabinose.

TABLE VII.8

ANALYTICAL DATA FOR *ACACIA XANTHOPHLOEA* GUM AND ITS DEGRADATION PRODUCTS

Polysaccharide	Optical rotation* (degrees)	N, (%)	Periodate* reduced after 72 hours (m moles/g)	Formic acid* released after 72 hours (m moles/g)	Molecular* weight ($x10^3$)
Acacia xanthophloea gum	+35	0.09	10.6	1.5	860
Degraded gum A	+18	0.19	23.4	4.5	320
Degraded gum B	-6	0.83	n.d.	n.d.	n.d.
Polysaccharide I	-18	0.4	12.1	1.3	210
Polysaccharide II	-7	1.12	11.9	2.4	33
Polysaccharide III	-17	2.88	n.d.	n.d.	n.d.

* Corrected for moisture.

n.d. not done.

Separation of a large-scale 0.5M acid hydrolysate (Duolite A-4 ion exchange resin column) into neutral and acidic fractions showed the presence of three aldobiuronic acids. One of them was positively identified by chromatography and methylation studies as 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose. The second one was characterised by paper chromatography (R_{gal} 0.62 solvent (a)) by specific rotation ($[\alpha]_D$ = +91°), and for hydrolysis studies as 4 - O - (4 - O - methyl - α - D - glucopyranosyluronic acid) - D - galactose. The third acidic component was chromatographically identical to 6 - O - (4 - O - methyl - β - glucuronic acid) - D - galactose, it was expected since A. xanthophloea gum has a high proportion of methoxyl content.

A. xanthophloea gum was methylated. Methanolysis followed by g.l.c. examination showed the presence of 2,3,4 - tri - O - methyl - L - rhamnose, 2,3,5 - and 2,3,4, - tri - , 2,5 - , 3,4 - and 3,5 - di - O - methyl - L - arabinose, 2,3,4,6 - tetra -, 2,3,6 - , 2,4,6 - and 2,3,4 - tri, 2,4 - di - O - methyl - D - galactose, and 2,3,4 - tri - O - methyl - D - glucuronic acid. The presence of small amounts of 2 - O - methyl - D - galactose was also observed, but its structural significance is doubtful as it may arise from either incomplete methylation or from demethylation during hydrolysis. The presence of 2,4 - di - O - methyl D - galactose indicates that some of the galactose residues are 3,6 - di - O - substituted, the identification of trace amounts of 2,3,6 -, 2,4,6 - , and 2,3,4 - tri - O - methyl - D - galactose is evidence for some 4 - O -, 3 - O -, and 6 - O - substituted D - galactose units respectively; and the presence of 2,3,4,6 - tetra - O - methyl - D - galactose is evidence for the presence of non-reducing D - galactose residues.

The identification of 2,3,4 - tri - O - methyl - D - glucuronic and methyl - L - rhamnose indicates that the L - rhamnose and glucuronic acid units occur as non-reducing end groups in A. xanthophloea as is customary in Acacia gum exudates. The di - O - methyl - arabinoses identified were 2,5 - , (11 parts), 3,4 - , (7 parts) and the less common 3,5 - di - O - methyl - L - arabinose (one part). The identification of 2,5 - di - O - methyl - L - arabinose is evidence for some 3 - O - substituted L - arabinofuranose residues and the identification of 3,4 -, and 3,5 - di - O - methyl - L - arabinose indicates that L - Arabinopyranosyl - (1 $\longrightarrow$ 2) - L - arabinose and L - Arabinofuranosyl - (1 $\longrightarrow$ 2) - L - arabinose must be present in the gum. The presence of 2,3,5 - and 2,3,4 - tri - O - methyl - L - arabinose indicates that the arabinose - containing side-chains are terminated in some cases by L - arabinofuranose and in other cases by L - arabinopyranose residues.

Hydrolysis of A. xanthophloea gum with 0.01N sulphuric acid for 96 hours on a boiling water bath gave degraded gum A (38.3% yield). The distilled water dialysate from the preparation of degraded gum A was concentrated and examined chromatographically. Large amounts of arabinose were detected along with smaller amounts of galactose and rhamnose. Also liberated were 6 - O - β - D - glucopyranosyl - D - galactose and 3 - O - β - D - arabinopyranosyl - L - arabinose and a β - D - (1$\longrightarrow$ 6) linked galactose trisaccharide. The β - D - (1$\longrightarrow$ 3) - linked trisaccharide was detected in A. senegal gum (2) and the β - D - (1$\longrightarrow$ 6) - linked trisaccharide was detected in A. nubica gum

(6). A. xanthophloea gum therefore appears to be more similar to A. nubica gum (6) than to A. senegal gum in this respect.

Degraded gum A contained galactose (69%) as the major component, with arabinose (4%), rhamnose (8%) and uronic acid (19%); this increased proportion of uronic acid relative to that in the whole gum arises because 0.01N acid hydrolysis does not break uronosyl linkages. Partial hydrolysis showed 6 - O - β - D - galactopyranosyl - D - galactose to be the major galactobiose present, a fact confirmed by the presence of large amounts of 2,3,4 - tri - O - methyl - D - galactose in the methanolysate of degraded gum A.

Methylated degraded gum A, on methanolysis and hydrolysis, gave 2,3,4,6 - tetra - , 2,3,4 - , 2,4,6 - , and 2,3,6 - tri - , and 2,4 - di - O - methyl - D - galactose, 3,5 - , and 2,5 - di - O - methyl - L - arabinose, 2,3,4 - tri - O - methyl - D - glucuronic acid, 2,3,4 - tri - O - methyl - L - rhamnose and trace amounts of 2,3,4 - , and 2,3,5 - tri - O - methyl - L - arabinose.

Degraded gum A was Smith-degraded to give degraded gum B. Degradation was extensive and a very small yield (5.3%) of degraded gum B was obtained because the large number of 1,6 - linked galactose residues in degraded gum A are periodate-vulnerable. Degraded gum B consisted of galactose (100%) but ca. 0.8% nitrogen was present and this may have arisen from a resistant polypeptide in an interior part of the original gum molecule. Certain evidence for this has been obtained recently by other workers (7).

A. xanthophloea gum was subjected to successive Smith-degradations, involving periodate oxidation, borohydride reduction, and controlled acid hydrolysis. Polysaccharide I contained galactose (70%), arabinose (26%), a trace of rhamnose, and uronic acid (4%); acid hydrolysis yielded a small amount of 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose. Thus the first Smith-degradation removed all the rhamnose and 4 - O - methyl-glucuronic acid residues together with some of the galactose, arabinose and glucuronic acid. In addition the residual % N (0.4%) was higher than in the whole gum and this may be due to the presence of a resistant polypeptide as was explained for degraded gum B. Non-reducing end-group arabinose and galactose, as well as 6 - O - substituted galactose residues and 4 - O - substituted glucuronic acid residues, should be cleaved during the first Smith-degradation. Methylation evidence for the whole gum indicates that all the glucuronic acid residues are present as non-reducing end-groups, and complete oxidation of all glucuronic acid residues would therefore be expected during the first treatment with periodate; slightly incomplete oxidation was also observed by earlier workers who considered the effect to have arisen from steric hindrance (8). Acetal linkages involving acid fragments are, however, difficult to hydrolyse with cold dilute acid (9) but as all the glucuronic acid residues are glycosidically linked to the C - 6 positions of galactose residues those acidic units resisting the first Smith-degradation are eliminated subsequently.

On methanolysis, the O - methyl derivative of polysaccharide

I gave the methyl glycosides (see Table VII.5) of 2,3,4 - tri - O - methyl - L - rhamnose, 2,3,5 - tri - , 2,5 - , and 3,5 - di - O - methyl - L - arabinose, 2,3,4,6 - tetra -, 2,4,6 - and 2,3,4 - tri -, 2,4 - di - O - methyl - D - galactose and 2 - O - methyl - D - galactose, and 2,3,4 - tri - O - methyl - D - glucuronic acid. Thus all arabinopyranose and most rhamnopyranose residues are removed by one Smith degradation. The presence of 2 - O - methyl - D - galactose may result from under methylation, at the C - 4 position, of some galactose residues in sterically hindered internal position of the molecule.

A second Smith-degradation yielded Polysaccharide II, which contained galactose (90%) and arabinose (10%). The nitrogen content (1.1%) was higher than in Polysaccharide I.

On methanolysis, the O - methyl derivative of Polysaccharide II gave the methyl glycosides of 3,5 and 2,5 - di - O - methyl - L - arabinose, and 2,4,6 - and 2,3,4, - tri - and 2,4 - di - O - methyl - D - galactose.

A Smith-degradation of Polysaccharide II gave Polysaccharide III, which contained only galactose (100%) and a residual nitrogen content (2.9%) higher than in Polysaccharide II. Partial hydrolysis of polysaccharides II and III followed by chromatography showed the presence of 6 - O - β - D - galactopyranosyl - D - galactose, 3 - O - β - D - galactopyranosyl - D - galactose and sequences of three β - 1,6 - linked galactose units were indicated. The most predominant O - methyl sugar obtained from methylated polysaccharide II was 2,4,6 - tri - O - methyl - D - galactose so that 1,3 - linkages are predominant, but the identification of 2,3,4 - tri - O - methyl - D

- galactose (Table VII.7) confirms that some β - 1,6 - linkages are also present. Three Smith-degradations were required to remove all the arabinose, indicating that some of the arabinose side chains were three units long.

The core of A. xanthophloea gum appears to be a highly branched galactan framework; some branches are terminated by D - glucuronic acid (and its 4 - O - methyl derivative) linked mainly β - (1 $\longrightarrow$ 6) to D-galactose, but with some α - (1 $\longrightarrow$ 4) links also. The linkages detected between the D - galactose residues were mostly β - (1 $\longrightarrow$ 6), with some β - (1 $\longrightarrow$ 3) also present. There was some evidence of three contiguous β - (1 $\longrightarrow$ 6) - linked D - galactose residues but no evidence for a "backbone" of (1 $\longrightarrow$ 3) - linked D - galactose residues. These results are in agreement with those found for the gums from A. nubica (6), A. senegal (2) and A. arabica (5).

A. xanthophloea gum gave a degraded gum consisting of D - galactose residues linked mainly β - (1 $\longrightarrow$ 6) with some β - D - (1 $\longrightarrow$ 3) linkages also present; a similar situation was reported for A. nubica gum (6) (Gummiferae) but not for A. senegal gum (2) (Vulgares).

As in A. seyal gum (10) (Gummiferae) the L - arabinose chains in A. xanthophloea gum are attached to either the C - 3 or C - 6 positions of D - galactose. In A. senegal gum (Vulgares) the arabinose units are attached to C - 3 of D - galactose; in A. pycantha gum (Phyllodineae) (11) they are attached to C - 6 of D - galactose. The L - arabinose chains in A. xanthophloea gum are shorter than in the gums from A. nubica and A. senegal

(where five and four Smith-degradations respectively were required to eliminate all of the L - arabinose). The short L- arabinose chains also differ from those of A. senegal and A. nubica gums in that they consist largely of (1 ⟶ 2) linked L - arabinofuranose units although such chains have been reported to occur in the gums from A. pycnantha (11), A. arabica (5), and other Acacia species (12).

The structural evidence suggests:

a) The core of A. xanthophloea gum appears to be a branched galactan framework; some branches are terminated by D - glucuronic acid and some by its 4 - O - methyl derivative.

b) The only linkages detected between the D - galactose residues were β - (1 ⟶ 3) and β (1 ⟶ 6) with the latter type preponderating.

c) Chains of L - arabinose residues are attached to the branched galactan framework at either the C - 3 or C - 6 positions of D - galactose; the L - arabinose chains are at least three units long, and some are terminated by L - arabinofuranose and L - arabinopyranose residues linked β - (1 ⟶ 3) to L - arabinose. The chains contain (1 ⟶ 2) - linked L - arabinofuranose and L - arabinopyranose residues, and a smaller proportion of (1 ⟶ 3) linkages.

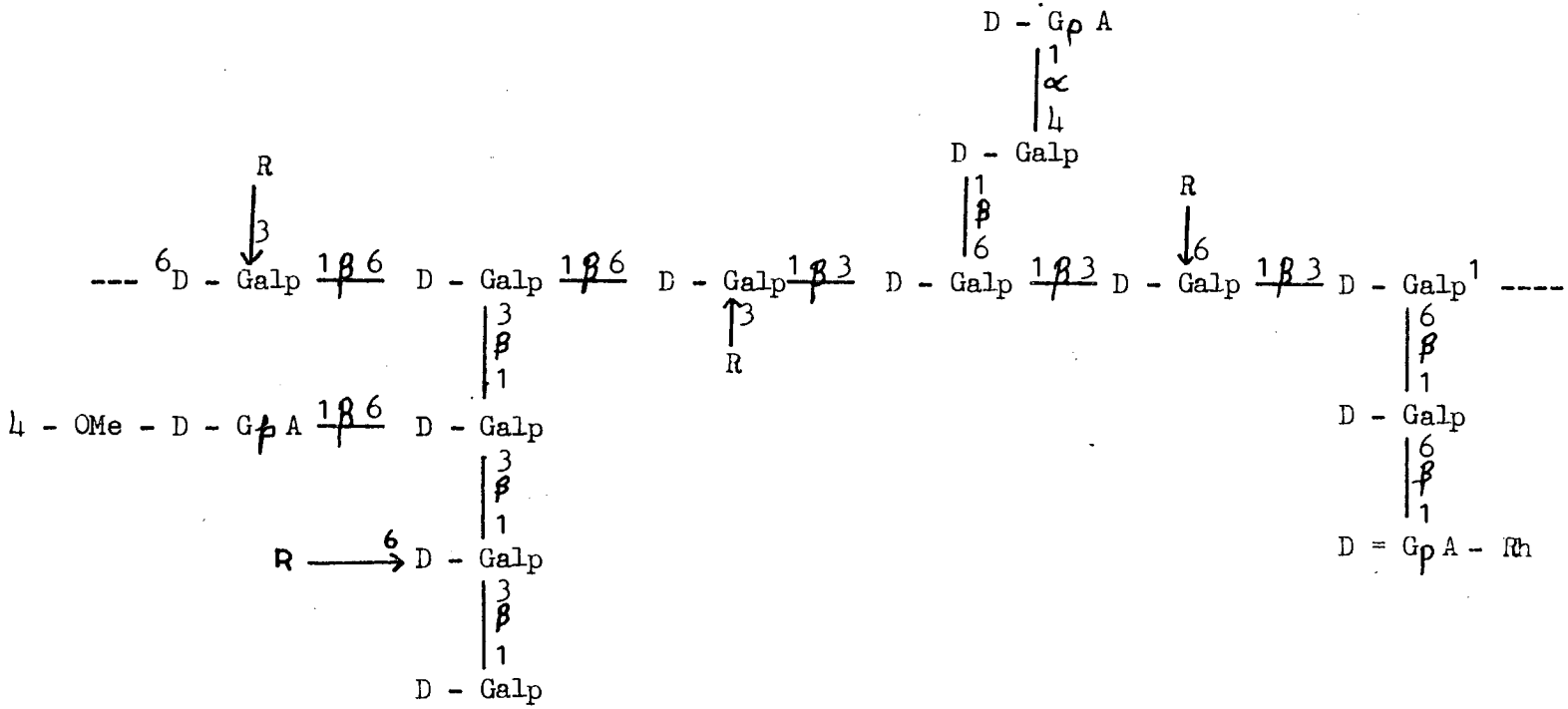
d) L - rhamnose residues occur as non-reducing end groups.

e) The nitrogen contents of all the degradation and periodate-oxidised products are higher than that of the whole gum and this may indicate the presence of a resistant polypeptide or protein in an interior part of the original gum molecule.

On the basis of the available evidence a possible structural

model for Acacia xanthophloea gum molecule is given in Fig. VII.3.

Fig. VII.3 Possible structural model for Acacia xanthophloea gum



R represents L - Arap and L - Araf - containing sidechains. Some of these side-chains are at least three units long and contain ...^{3}L - Araf^{1} .. L^{3} - Arap^{1} ..., L^{2} - Araf^{1} and L^{2} - Arap^{1} residues.

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SECTION VIII

NATURAL ABUNDANCE ^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF *A. XANTHOPHLOEA* GUM AND ITS DEGRADATION PRODUCTS

VIII.1 INTRODUCTION

Carbon-13 nuclear magnetic resonance is rapidly becoming one of the most efficient spectroscopic methods for configurational and conformational investigations in carbohydrate chemistry. Although ^{13}C- n.m.r. has been applied extensively to monosaccharides (1-3) and oligosaccharides (3,4), its potential in determining polysaccharide structure has not been explored to any great extent. As the technique becomes better understood and more accurate it is being more frequently used for elucidation of the composition, sequence, and conformation of polysaccharides (5 - 23).

The method depends heavily however, on the availability of the relevant model compounds; ^{13}C- n.m.r. spectra for some model compounds, e.g. D - glucopyranosyl-L-- rhamnopyranose and D - galactopyranosyl - L - rhamnopyranose disaccharides having various types of linkage and of some disaccharides containing 2 - acetamido - 2 - deoxy - D - glucose or 2 - acetamido - 2 - deoxy - D - galactose, have been reported (23). There have also been studies on glucans (10), mannans (17) and hyaluronic acid (20); these polymers have mono- or - disaccharide repeating units.

Recently, studies of heteropolysaccharides have begun with the assignment of signals to disaccharides required as models for subsequent studies of inmunopolysaccharides (22); the spectra of various chondroitin sulphate molecules have been recorded (23).

^{13}C - n.m.r. spectra of the gum from Acacia auriculiformis and its degradation products have been recorded (24) and an attempt has been made to assign signals.

The present study is concerned with ^{13}C - n.m.r. spectra

TABLE VIII.1 *

ASSIGNMENT OF THE ^{13}C RESONANCES+ OF DISACCHARIDES OF THE D-GALACTOSYL-L-RHAMNOSE SERIES

	D-Galactose	L-Rhamnose	4-O-β-D-Galactopyranosyl-L-rhamnopyranose	3-O-β-D-Galactopyranosyl-L-rhamnopyranose	2-O-β-D-Galactopyranosyl-L-rhamnopyranose[c]
C-1	-	-	104.9	105.5	105.9(α) 105.1(β)
C-1α	93.4	95.2	95.0	95.0	94.1
C-1β	97.7	94.7	94.6	94.5[b]	93.9
C-2	-	-	72.9	72.4[b]	72.2
C-2α	70.3[a]	72.0	72.0	71.9[b]	81.7
C-2β	73.0	72.5	72.5[b]	72.4[b]	82.4
C-3	-	-	74.0[b]	73.8	73.7
C-3α	69.4	71.2	71.2	81.0	71.1
C-3β	73.9	74.0	74.0[b]	83.4	74.2
C-4	-	-	69.8	69.9[b]	69.7
C-4α	70.4[a]	73.4	82.3	72.4[b]	73.6[b]
C-4β	69.8	73.2[b]	81.9	72.4[b]	73.3
C-5	-	-	76.4	76.3	76.2
C-5α	71.6	69.5	68.1	69.5	69.3
C-5β	76.2	73.2[b]	71.8	73.0	73.6[b]
C-6	-	-	62.1	62.3	62.2
C-6α	62.3	18.0[b]	18.2[b]	18.1[b]	18.1
C-6β	62.1	18.0[b]	18.2[b]	18.1[b]	17.9

* Reproduced from ref. 23.

\+ Chemical shifts values in p.p.m. relative to TMS = o.

TABLE VIII. 2*

^{13}C CHEMICAL SHIFTS+ OF GALACTOSE AND ITS DERIVATIVES

Compound	C - 1	C - 2	C - 3	C - 4	C - 5	C - 6
α - D - Galactopyranose	92.35	69.40	68.40	69.20	70.50	61.25
β - D - Galactopyranose	96.50	71.90	72.85	68.80	75.20	61.05
Methyl - α - D - galactopyranoside	99.50	69.60	68.30	69.30	70.80	61.30
Methyl - β - D - galactopyranoside	103.90	70.80	72.85	68.75	75.20	61.05
Methyl - 3 - O - methyl - β - D - galactopyranoside	103.90	69.80	82.00	64.20	75.10	61.20
Methyl - 2,6 - O - dimethyl - α - D - galactopyranoside	96.80	77.45	68.60	69.50	73.15	71.85
Methyl - 2,3,4,6 - tetra - O - methyl - β - D - galactopyranoside	103.35	79.75	82.20	73.05	74.90	71.00

* Reproduced from ref. 25.

\+ Chemical shifts values in p.p.m. relative to TMS = o

of the gum from Acacia xanthophloea and its degradation products; some assignments of signals have been possible on the basis of the structural study described in Section VII and from the results of previous workers (1 - 25).

Acacia xanthophloea gum contains galactose, arabinose, rhamnose, glucuronic acid and 4 - O - methylglucuronic acid.

Signal assignment in the spectra of polysaccharides is considerably simplified by the fact that ^{13}C - n.m.r. resonances fall into certain well-defined regions, regardless of the identity of the sugar involved (22). Tables VIII.1 and VIII.2 show the chemical shifts reported (22, 25) for galactose and its derivatives.

VIII.2 RESULTS AND DISCUSSION

Carbon-13 magnetic resonance spectra were obtained from solutions in deuterium oxide of Acacia xanthophloea gum, and from its degraded gum A, degraded gum B, and its derived polysaccharides I, II and III. The spectra obtained are given in Figures II to VII.

The natural abundance ^{13}C - n.m.r. spectrum of Acacia xanthophloea gum (Fig. II) is extremely complex. This was expected as the gum contains five different sugars and has a high molecular weight. Nevertheless, there are four well-defined regions in the spectrum:-

a) the first has a prominent peak at δ = 60.9 p.p.m., which is probably from the methoxyl group in 4 - O - methyl - D - glucuronic acid. This peak disappears in later spectra after Smith-degradation. Also there is a small peak at

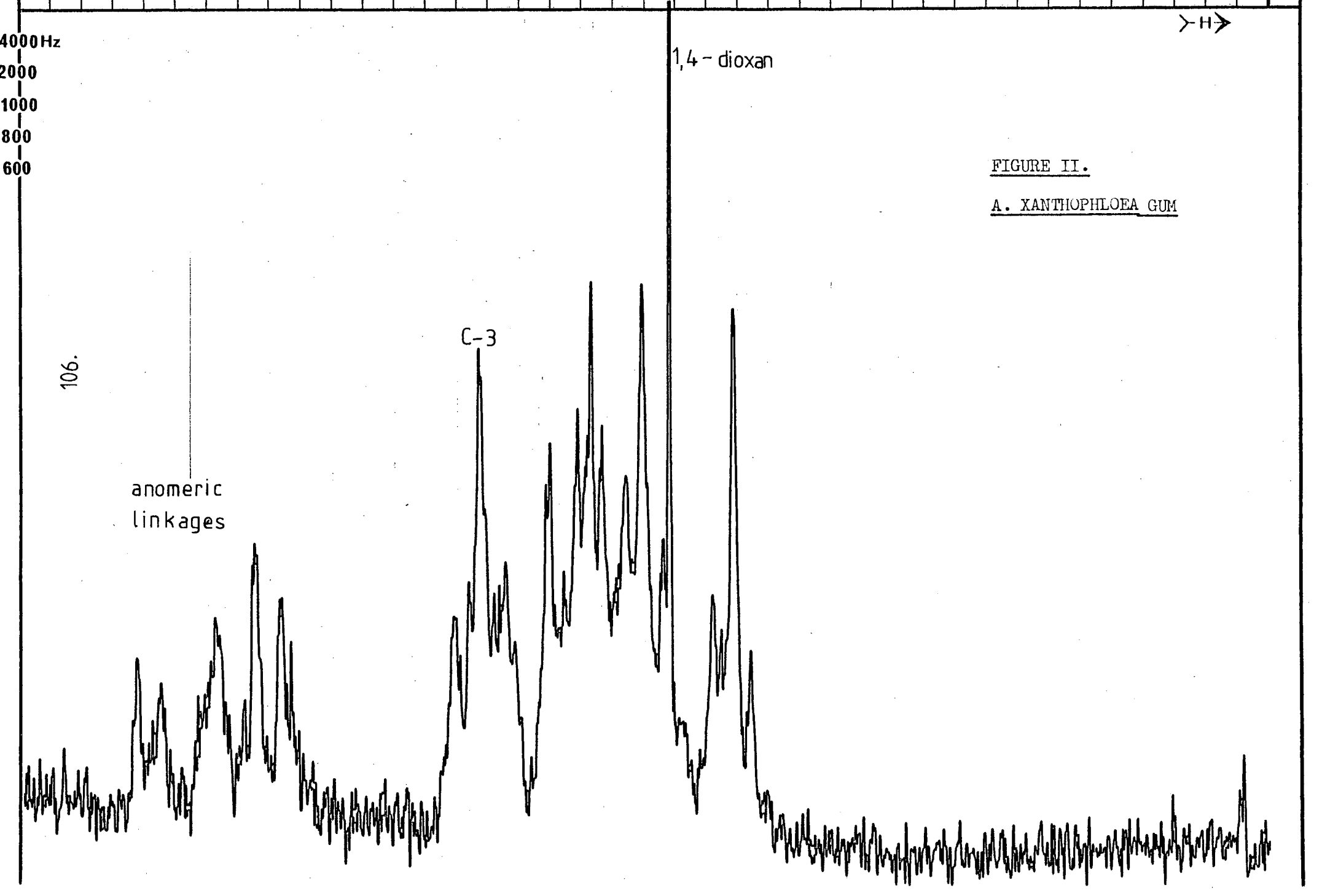
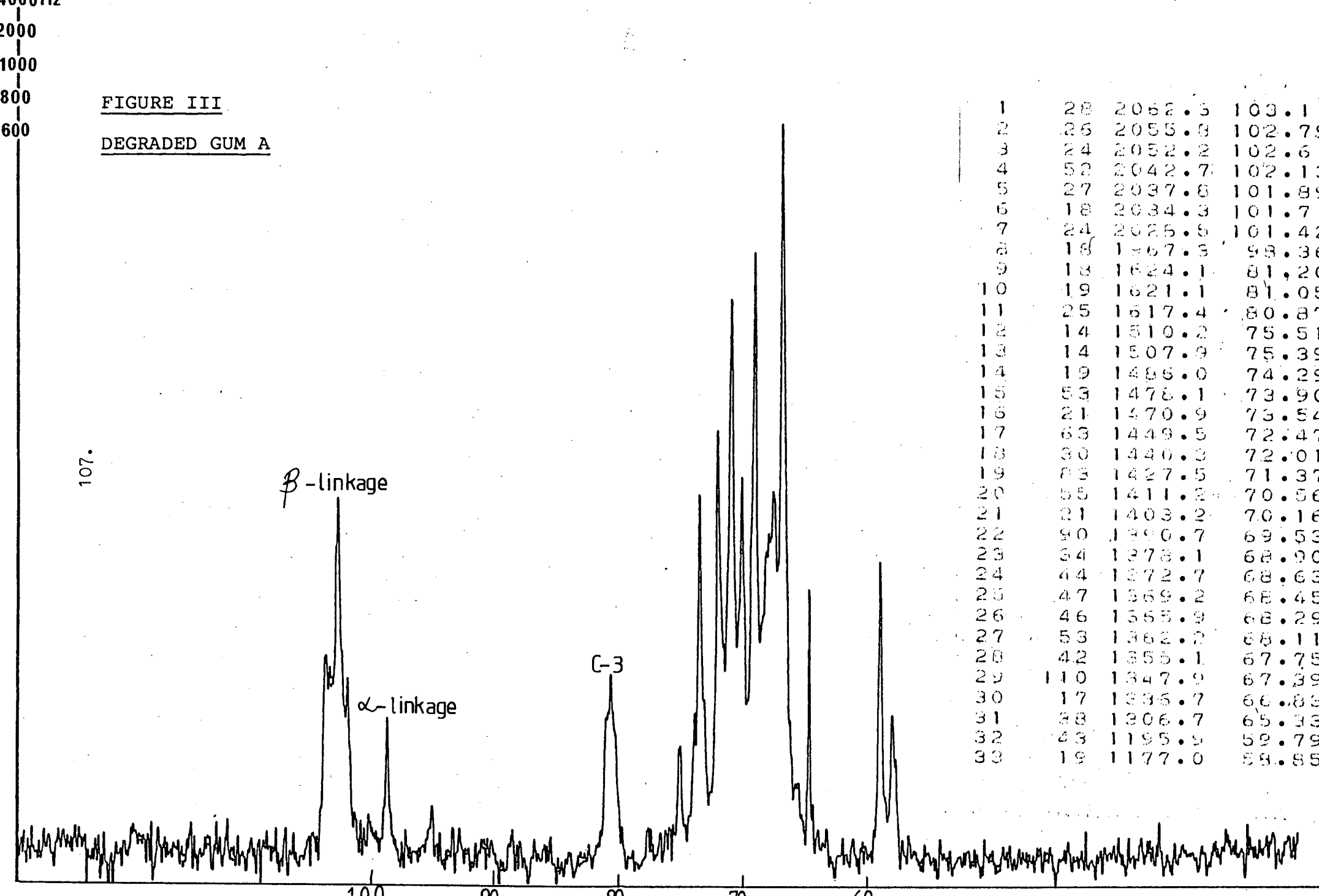


FIGURE II.

A. XANTHOPHLOEA GUM

FIGURE III

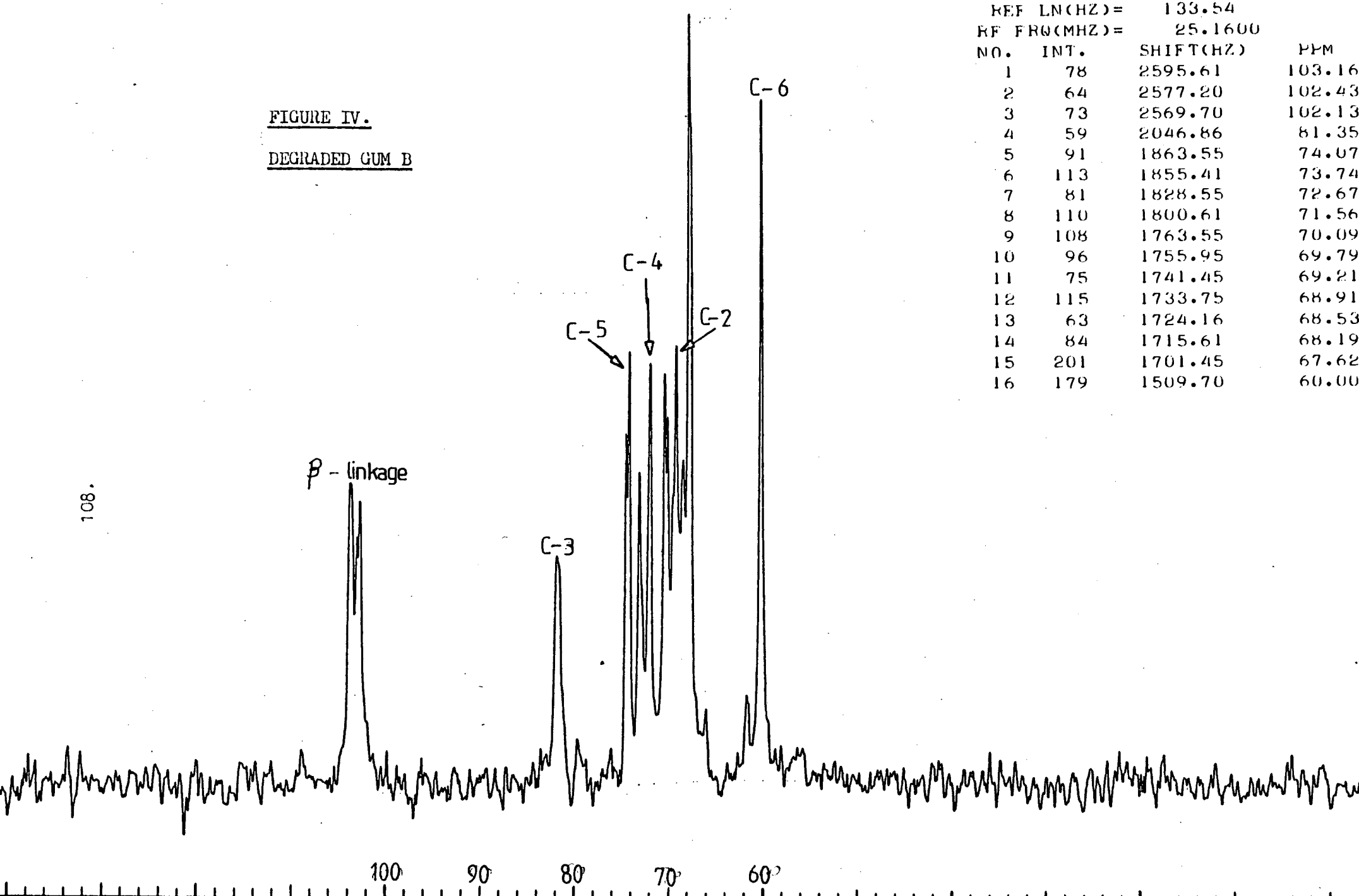
DEGRADED GUM A



1	28	2062.5	103.1
2	26	2055.8	102.79
3	24	2052.2	102.6
4	52	2042.7	102.13
5	27	2037.8	101.89
6	18	2034.3	101.7
7	24	2025.8	101.42
8	18	1967.3	98.36
9	13	1624.1	81.20
10	19	1621.1	81.05
11	25	1617.4	80.87
12	14	1510.2	75.51
13	14	1507.9	75.39
14	19	1485.0	74.25
15	53	1476.1	73.80
16	21	1470.9	73.54
17	63	1449.5	72.47
18	30	1440.3	72.01
19	28	1427.5	71.37
20	55	1411.2	70.56
21	21	1403.2	70.16
22	90	1390.7	69.53
23	54	1378.1	68.90
24	44	1372.7	68.63
25	47	1369.2	68.45
26	46	1365.9	68.29
27	53	1362.2	68.11
28	42	1355.1	67.75
29	110	1347.9	67.39
30	17	1335.7	66.83
31	38	1306.7	65.33
32	43	1195.5	59.79
33	19	1177.0	58.85

FIGURE IV.

DEGRADED GUM B



THRESH(CM)=	3.0
REF LN(HZ)=	133.54
RF FRQ(MHZ)=	25.1600

NO.	INT.	SHIFT(HZ)	PPM
1	78	2595.61	103.16
2	64	2577.20	102.43
3	73	2569.70	102.13
4	59	2046.86	81.35
5	91	1863.55	74.07
6	113	1855.41	73.74
7	81	1828.55	72.67
8	110	1800.61	71.56
9	108	1763.55	70.09
10	96	1755.95	69.79
11	75	1741.45	69.21
12	115	1733.75	68.91
13	63	1724.16	68.53
14	84	1715.61	68.19
15	201	1701.45	67.62
16	179	1509.70	60.00

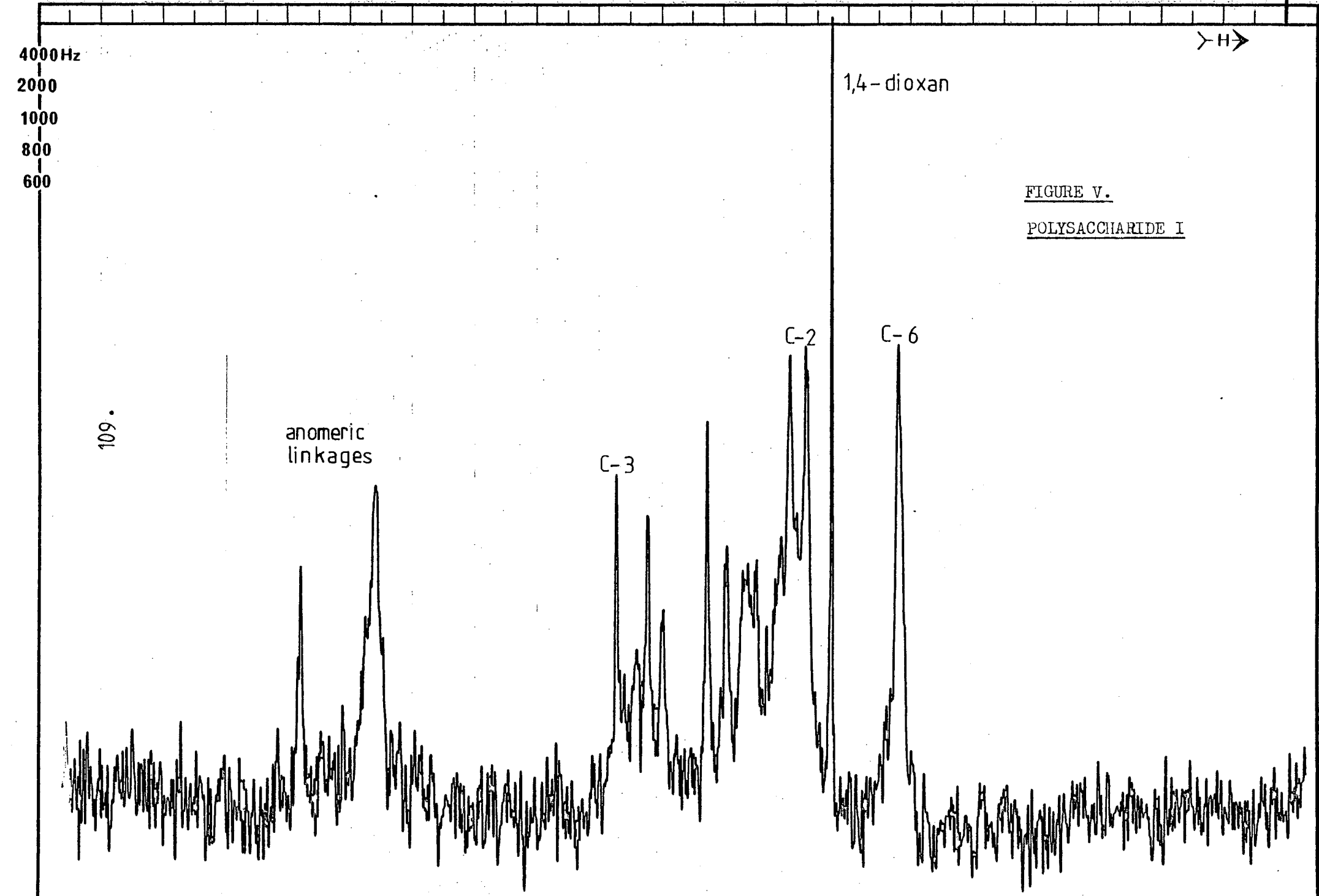


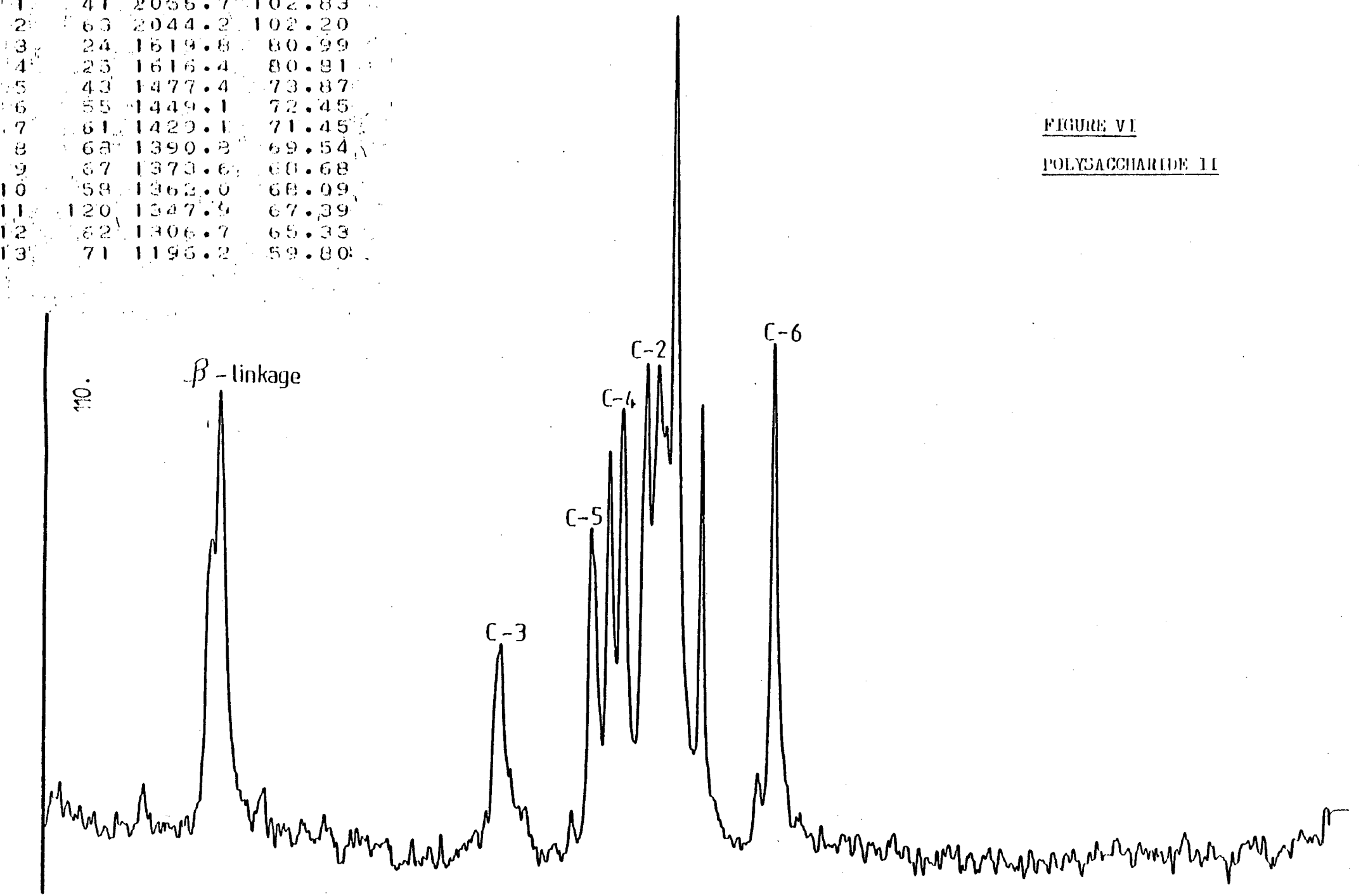
FIGURE V.

POLYSACCHARIDE I

1	41	2056.7	102.83
2	63	2044.2	102.20
3	24	1619.8	80.99
4	23	1616.4	80.81
5	43	1477.4	73.87
6	55	1449.1	72.45
7	61	1429.1	71.45
8	68	1390.8	69.54
9	67	1373.6	68.68
10	58	1362.0	68.09
11	120	1347.9	67.39
12	62	1306.7	65.33
13	71	1196.2	59.80

FIGURE VI

POLYSACCHARIDE II

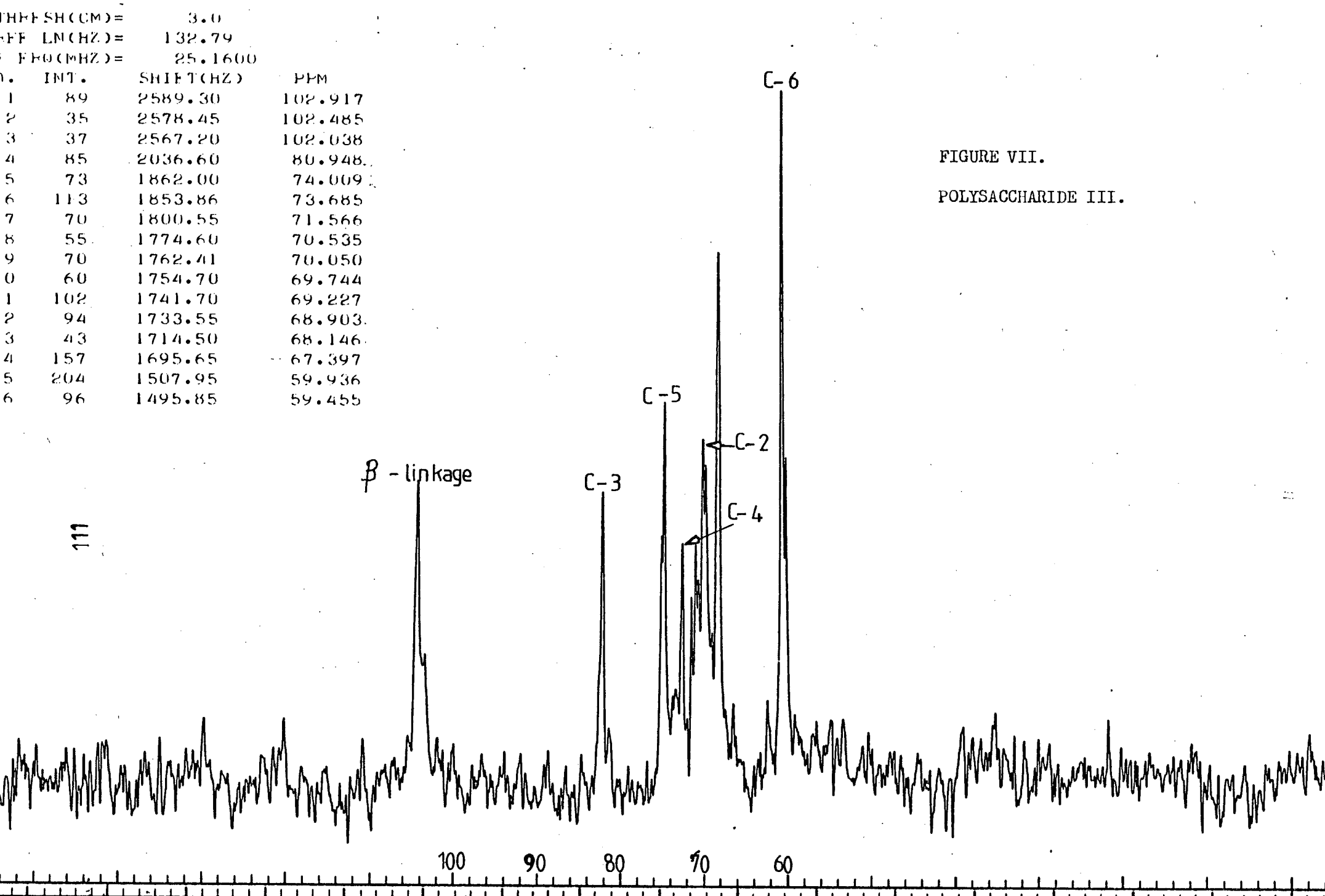


THRESH(CM)= 3.0
FF LN(HZ)= 132.79
FRQ(MHZ)= 25.1600

.	INT.	SHIFT(HZ)	PPM
1	89	2589.30	102.917
2	35	2578.45	102.485
3	37	2567.20	102.038
4	85	2036.60	80.948
5	73	1862.00	74.009
6	113	1853.86	73.685
7	70	1800.55	71.566
8	55	1774.60	70.535
9	70	1762.41	70.050
0	60	1754.70	69.744
1	102	1741.70	69.227
2	94	1733.55	68.903
3	43	1714.50	68.146
4	157	1695.65	67.397
5	204	1507.95	59.936
6	96	1495.85	59.455

FIGURE VII.

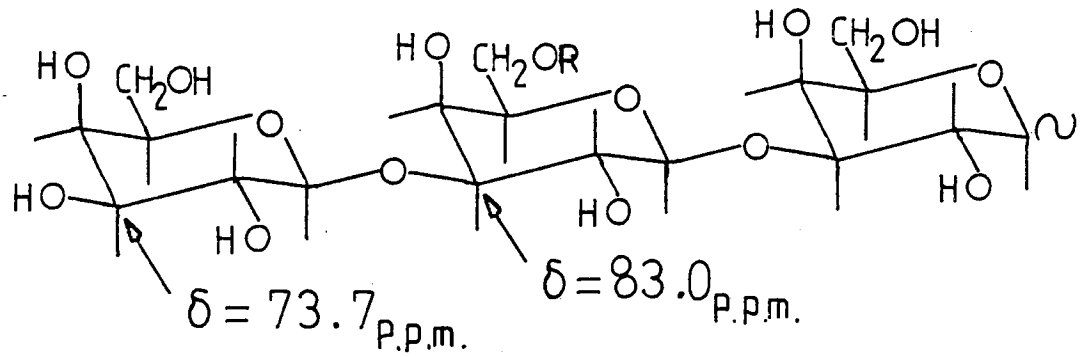
POLYSACCHARIDE III.



δ = 61.4 p.p.m., probably from the hydroxylated C-6 of galactose as reported by other workers (23).

b) The second region contains the peaks between δ= 67 p.p.m. and 77 p.p.m. These are the resonances of the hydroxylated ring carbon atoms. This region is far too complex to be of any practical significance. The large peak at δ= 67.4 is due to 1,4-dioxan, added as an internal reference.

c) The group of peaks centred at δ= 83 p.p.m. is due to glycosidically-linked C-3 of galactose. The presence of these resonances was expected, as A. xanthophloea gum contains β - 1,3 linked galactose residues (Section VII). The multiplicity of peaks in this region reveals several different environments within the molecule. The diagram[1] shows the case.



d) The resonances of the anomeric carbon atoms lie in the region δ= 100 - 105 p.p.m. This range is wider in this spectrum (100 - 119 p.p.m.) and it shows the presence of at least five different types of linkage. This region becomes simpler in the spectra of the degraded products. A peak at 100.5 p.p.m. is probably due to an α - linkage; it has been shown that A. xanthophloea gum contains some α - linkages (Section VII). The group of peaks centred on δ= 104.3 p.p.m. is almost certainly due to the β - linked C-1 of galactose. Smaller peaks in the same region may be due to the anomeric carbon atoms of other sugars present. It has been shown (Section VII) that the majority of glycosidic linkages in the gum are β- linkages.

Natural abundance ^{13}C - n.m.r. spectrum of degraded gum A (Fig. III)

Degraded gum A, obtained by mild acid hydrolysis of A. xanthophloea gum (See Section VII), contains galactose, arabinose, rhamnose and uronic acid. During the hydrolysis large quantities of arabinose, especially acid-labile arabinofuranose residues, were removed from the whole gum.

This spectrum (Fig. III) is complex but simpler than that of A. xanthophloea gum. The four main regions defined previously (Fig II) for the whole gum spectrum are still present and peak assignment is therefore a more realistic operation:

a) The peak at δ= 59.8 p.p.m. is due to the hydroxylated C-6 of galactose. It is interesting to note that the peak at 60.9 p.p.m. in Spectrum I, now appears at 59.8 p.p.m.

The presence of this peak is expected as the methoxyl content of degraded gum A is similar to that of A. xanthophloea gum.

b) The large peak at δ= 67.4 p.p.m. is due to the internal reference, 1,4- dioxan.

c) The resonances in the δ 67-75 p.p.m. region due to hydroxylated ring carbons have become more simplified. The most prominent peak (69.5 p.p.m.) is probably due to hydroxylated C-2 of galactose. The peak at δ= 65.3 p.p.m. is almost certainly due to glycosidically linked C-6 of galactose shifted downfield by 3 p.p.m. relative to the unsubstituted C 6 of galactose. As this resonance disappears in the spectrum of degraded gum B (Fig. IV) it may arise from a periodate-vulnerable entity.

d) The region at δ= 81.0 p.p.m. attributed to C-3 of glycosidically linked galactose, has also been simplified as the mild acid hydrolysis removed almost all the arabinose residues from the whole gum.

e) The number of peaks given by anomeric carbon atoms has decreased in the range from 98 p.p.m. to 103 p.p.m.; the majority of these appear to come from β -linked galactose (102-103 p.p.m.). This is in agreement with the results found by chemical methods. The smaller peak at δ= 101 p.p.m. may be due to the presence of an α - linkage. It is interesting to note that the peaks in the range 104-119 p.p.m. in the Spectrum of the whole gum (Fig. II) do not appear in this spectrum. They could, therefore, be due to β -linked arabinose as most of the arabinose residues in the original gum were removed in the preparation of degraded gum A.

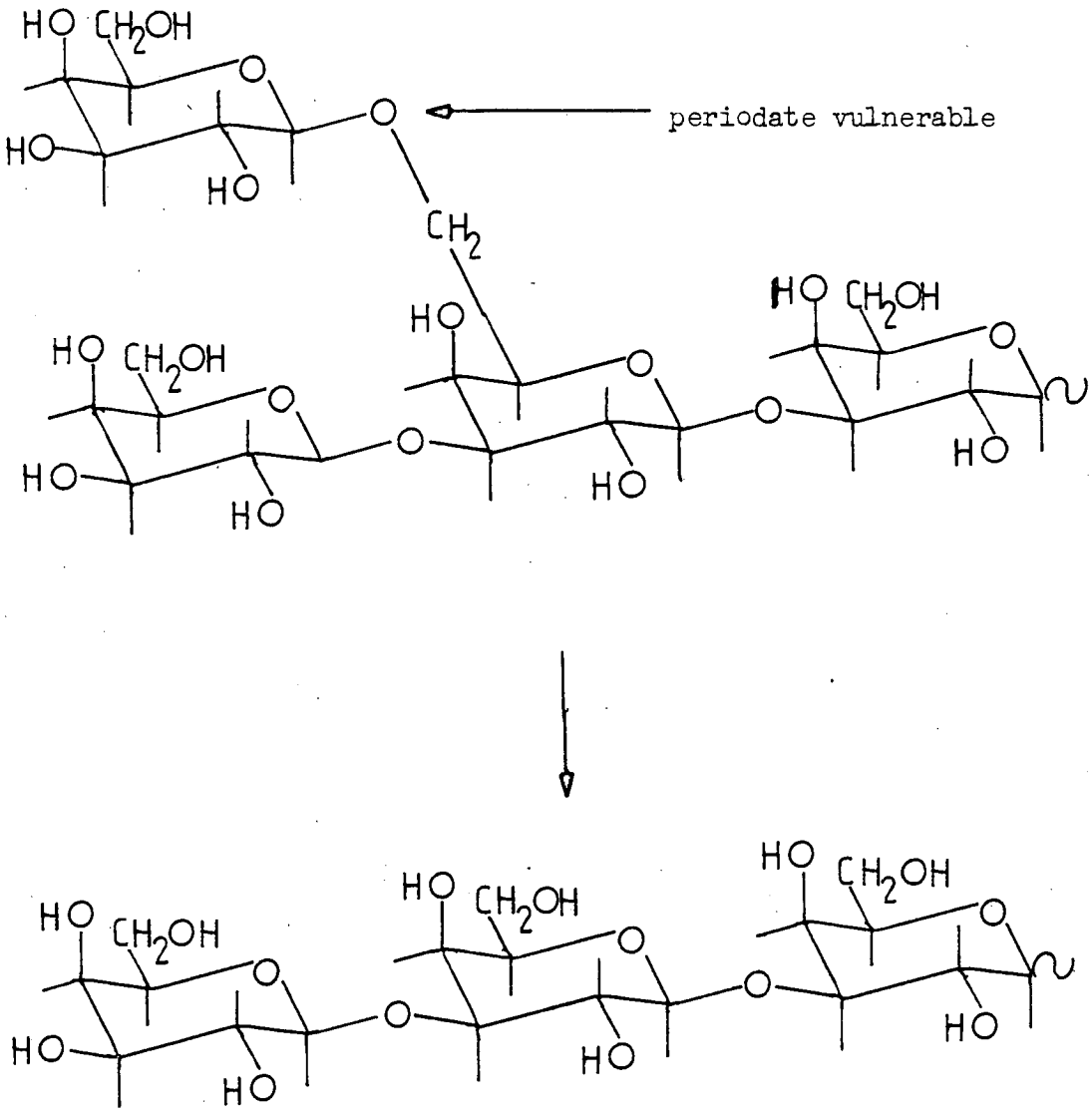
Natural abundance ^{13}C - n.m.r. Spectrum of degraded gumB (Fig IV)

Degraded gum B, obtained by Smith-degradation of degraded gum A (See Section VII) consists of galactose only.

This spectrum (Fig IV) is simpler than the previous ones (Figs II and III) and was expected because degraded gum B is basically a residual galactan after all the periodate - vulnerable other sugar residues have been removed. Analysis of this spectrum is made by comparison with that of methyl - 3 - O - methyl -β - D - galactopyranoside (Table VIII.2).

a) The large peak at δ=60.0 p.p.m. is assigned to the hydroxylated C - 6 of galactose. This peak has increased in size relative to the others throughout the degradation, and this is consistent with the removal of periodate - vulnerable side chains from the periodate - resistant galactan core. The diagram 2 shows the Smith-degradation.

Diagram 2 Smith-degradation



b) The large peak at δ= 67.6 p.p.m. is due to the internal reference 1,4 - dioxan.

c) The resonances of the hydroxylated carbon atoms of the sugar ring (δ= 67 - 74 p.p.m.) are much clearer in this spectrum than in those discussed previously. The three main peaks in this region are doublets whose separation is very small. Assignments for these peaks are still difficult. The most prominent peaks at δ= 68.5 p.p.m. and 74.0 p.p.m. are probably due to hydroxylated C-2 and C-5 of galactose by analogy with spectrum of methyl - 3 - O - methyl - β - D - galacto-pyranoside (Table VIII.2).

d) The peak assigned to glycosidically linked C-3 of galactose (δ= 81.3 p.p.m.) stands by itself in this spectrum. This suggests the presence of a simple chain of β- 1,3-linked galactose residues as there is only one environment for the carbon atom concerned. This interpretation is in agreement with the results found (See Section VII).

e) The resonances of the anomeric carbon atoms (102 - 103 p.p.m.) are very similar to those in the previous spectrum (Fig. III) indicating that all the galactose linkages are β in degraded gum B.

Natural abundance ^{13}C-n.m.r. Spectrum of polysaccharide I (Fig V)

Polysaccharide I, obtained by the periodate-oxidation, borohydride reduction, and subsequent mild acid hydrolysis of Acacia xanthophloea gum (See Section VII), contained galactose (70%), arabinose (26%), uronic acid (4%) and trace amounts of rhamnose.

The spectrum of polysaccharide I is less complicated than

that given by A. xanthophloea whole gum (Fig. II). It shows the four well-defined regions described previously:

a) The first region has a prominent peak at δ = 61.9 p.p.m. due to hydroxylated C - 6 of galactose. It is interesting to note that the peak which occurred at δ = 60.9 p.p.m. in the spectrum of A. xanthophloea gum (Fig. II) has disappeared. The methoxyl content of polysaccharide I was very low.

b) The resonances in the region 67 - 77 p.p.m., due to hydroxylated ring carbon atoms, is still far too complex to be of any practical significance. The large peak is due to the internal reference, 1,4 - dioxan.

c) The region 80 - 85 p.p.m., attributed to C - 3 of glycosidically linked galactose, is less complex. It is possible that the removal of periodate - vulnerable peripheral residues has reduced the number of environments.

d) The number of peaks in the region (102 - 129 p.p.m.) given by anomeric carbon atoms has decreased. The majority of the peaks belong to β - linked galactose.

Natural abundance ^{13}C - n.m.r. Spectrum of polysaccharide II. (Fig. VI).

Polysaccharide II, obtained by periodate-oxidation, borohydride reduction, and subsequent mild acid hydrolysis of polysaccharide I (See Section VII), contained only galactose (90%) and arabinose (10%).

The ^{13}C - n.m.r. spectrum appears to be simpler than that for polysaccharide I, as expected, because a large number of

vulnerable residues in the original structure has been removed.

The Spectrum shows that:-

a) The peak due to hydroxylated C - 6 of galactose at δ = 59.8 p.p.m. has increased in size relative to the other peaks. This indicates that a periodate-vulnerable residue is attached to the C - 6 of galactose in A. xanthophloea gum. Periodate-oxidation removes the vulnerable residues leaving galactose residues hydroxylated at C - 6. Diagram 2 illustrates this and shows how the relative number of hydroxylated C 6 atoms increases with periodate oxidation.

b) Although the region due to hydroxylated carbon atoms of the sugar ring (65 - 74 p.p.m.) is basically unchanged, it is simpler than that corresponding to the previous spectra (Figs. II, V).

c) The resonances due to glycosidically linked C - 3 of galactose at δ = 81 p.p.m. have become considerably simplified with only two overlapping peaks present. This reveals a reduction in the number of environments available to the C - 3 atom; (in fact there are 1,3 and 1,6 - linked galactose residues).

d) In the region where resonances due to anomeric carbon atoms occur there are two closely overlapping peaks; this is an indication that β - linked galactose occurs in at least two different environments. Methylation and hydrolysis studies (Section VII) revealed that polysaccharide II has both β - 1,3 and β -1,6 - galactose linkages.

The interpretation of the ^{13}C - n.m.r. spectrum of polysaccharide II (Fig.VI) indicates that this structure consists of a framework of β - 1,3 - linked galactose units with some substitution at their C - 6 position.

Natural abundance ^{13}C - n.m.r. Spectrum of polysaccharide III (Fig. VII).

Polysaccharide III, obtained by periodate oxidation, borohydride reduction, and subsequent mild acid hydrolysis of polysaccharide II (See Section VII) contains only galactose (100%).

The ^{13}C - n.m.r. spectrum is simpler than its predecessors, because the structure now contains galactose only. The ^{13}C- n.m.r. spectrum of polysaccharide III is very similar to that given by degraded gum B (Fig. IV) and this is not surprising as both are basically a chain of β - 1,3 - linked galactose units. It is interesting that the peak at δ= 59.9 p.p.m. due to the hydroxylated C - 6 of galactose has increased in size relative to the others throughout the degradation, and this is explained by the progressive removal of periodate-vulnerable side chains from the periodate resistant core (See diagram of Smith-degradation) (p116).

These studies have shown that valuable structural information can be obtained from ^{13}C - n.m.r. spectrum despite the complexity of these acid heteropolysaccharides of high molecular weight.

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SECTION IX

PROTON NUCLEAR RESONANCE SPECTROSCOPY OF *A. XANTHOPHLOEA* GUM AND ITS DEGRADATION PRODUCTS

IX.1. INTRODUCTION

Proton magnetic resonance (p.m.r.) spectroscopy has proved to be useful for the analysis of bacterial polysaccharides, and it has also been applied to determination of the structure of mucopolysaccharides (1-3).

The assignment of peaks in the p.m.r. spectra of polysaccharides is made on the basis of studies of the spectra of simple monosaccharides and oligosaccharides (4). The p.m.r. spectra of homopolysaccharides may be compared with the spectra for oligosaccharides of known structures in order to obtain information about the type of linkages involved (5).

The spectra of polysaccharides consist of:

a) a complex multiplet, due to the ring protons, at 3.5 - 4.6 p.p.m.

b) Anomeric proton resonances from 4.6 to 6.0 p.p.m. The position of the anomeric proton on C - 1 is unique and appears at higher frequency (4.5 - 6.0 p.p.m.) than the ring protons because the carbon to which it is attached bears two electron-withdrawing oxygen atoms.

c) Some substituents such as acetyl and pyruvate groups and the CH_3 - group of rhamnose resonate at frequencies not occupied by the ring protons; for example the CH_3 - group of rhamnose resonates at 1.7 p.p.m.

Most information is gained from the anomeric region, which indicates the number of sugar units in the repeating unit of the polysaccharide (6). Integration of the anomeric peaks and the substituent peaks gives the number of substituents in

each repeating unit of the polysaccharide (7,8). The peaks in the anomeric region are assigned after comparisons of the spectra of the lower oligosaccharides obtained from the polysaccharide with the spectrum of the polysaccharide itself (9).

P.m.r. spectra of carbohydrates are complex, with broad overlapping lines. An increasing number of chemical and physical methods is being developed to simplify spectra and to increase the resolution (10 - 14), although it is still not possible to make complete assignments of the spectra of polysaccharides.

A preliminary study of the p.m.r. spectra of gum exudates in deuterium oxide solution was carried out (15) to ascertain the usefulness of this technique in the determination of these complex acidic polysaccharides.

This study presents a series of p.m.r. spectra of Acacia xanthophloea gum and its degradation products.

IX.2. RESULTS AND DISCUSSION

The p.m.r. spectra were obtained for solutions, in deuterium oxide, of Acacia xanthophloea gum and for its degraded gums A and B and its derived polysaccharides I, II and III. The spectra obtained are given in Figures VIII to XIII.

The p.m.r. spectra of Acacia xanthophloea gum and its degradation products are very complex. All the spectra contain complex multiplets in the 4 - 4.6 p.p.m. region which arise from the resonances of the ring protons of the sugar units in the molecule. The peaks in the 4.7 - 5.7 p.p.m. region are due to the linked anomeric protons (4). There are at least five main

> SC

(HZ)=	576.7	5.764
(HZ)=	566.2	5.659
(HZ)=	563.9	5.636
(HZ)=	519.3	5.190
(HZ)=	481.7	4.814
(HZ)=	467.7	4.674
(HZ)=	447.0	4.468
(HZ)=	429.5	4.293
(HZ)=	399.9	3.997
(HZ)=	179.2	1.791
(HZ)=	173.3	1.732

125.

FIGURE VIII.

ACACIA XANTHOPHLOEA GUM

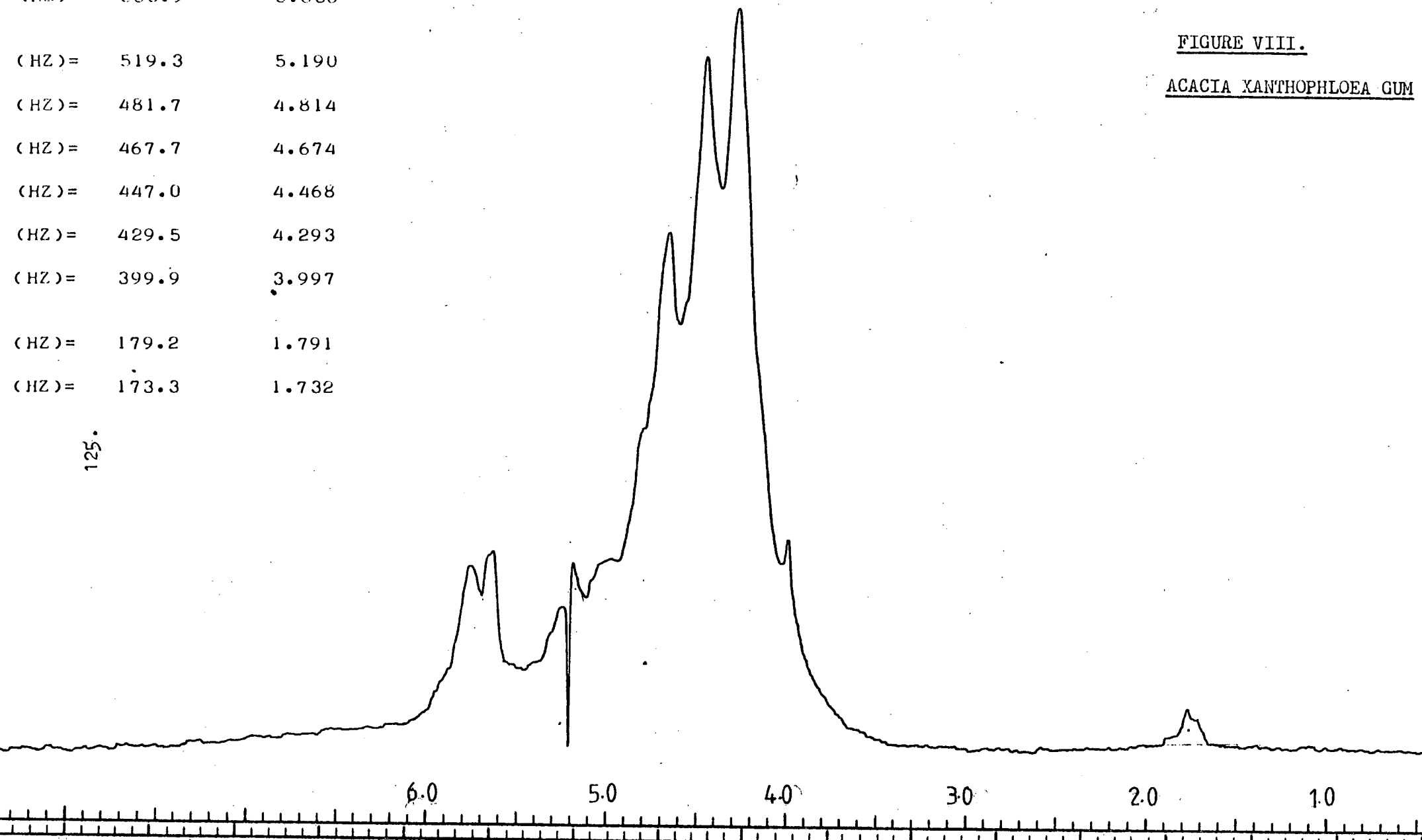
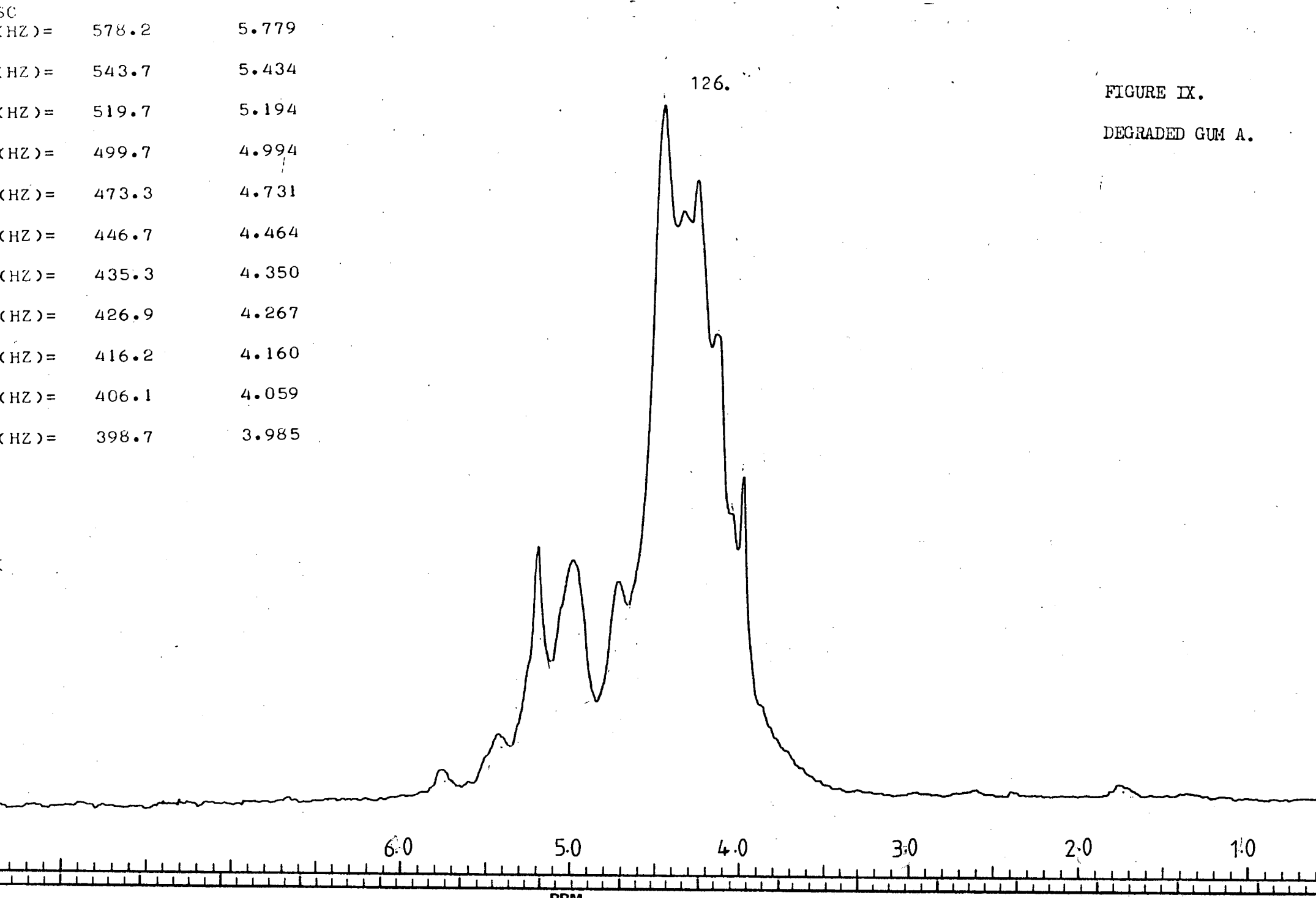


FIGURE IX.

DEGRADED GUM A.

SC		
(HZ)=	578.2	5.779
(HZ)=	543.7	5.434
(HZ)=	519.7	5.194
(HZ)=	499.7	4.994
(HZ)=	473.3	4.731
(HZ)=	446.7	4.464
(HZ)=	435.3	4.350
(HZ)=	426.9	4.267
(HZ)=	416.2	4.160
(HZ)=	406.1	4.059
(HZ)=	398.7	3.985



SC

(HZ)= 504.24 5.039

(HZ)= 468.89 4.686

(HZ)= 440.74 4.404

(HZ)= 422.64 4.223

FIGURE X.

DEGRADED GUM B.

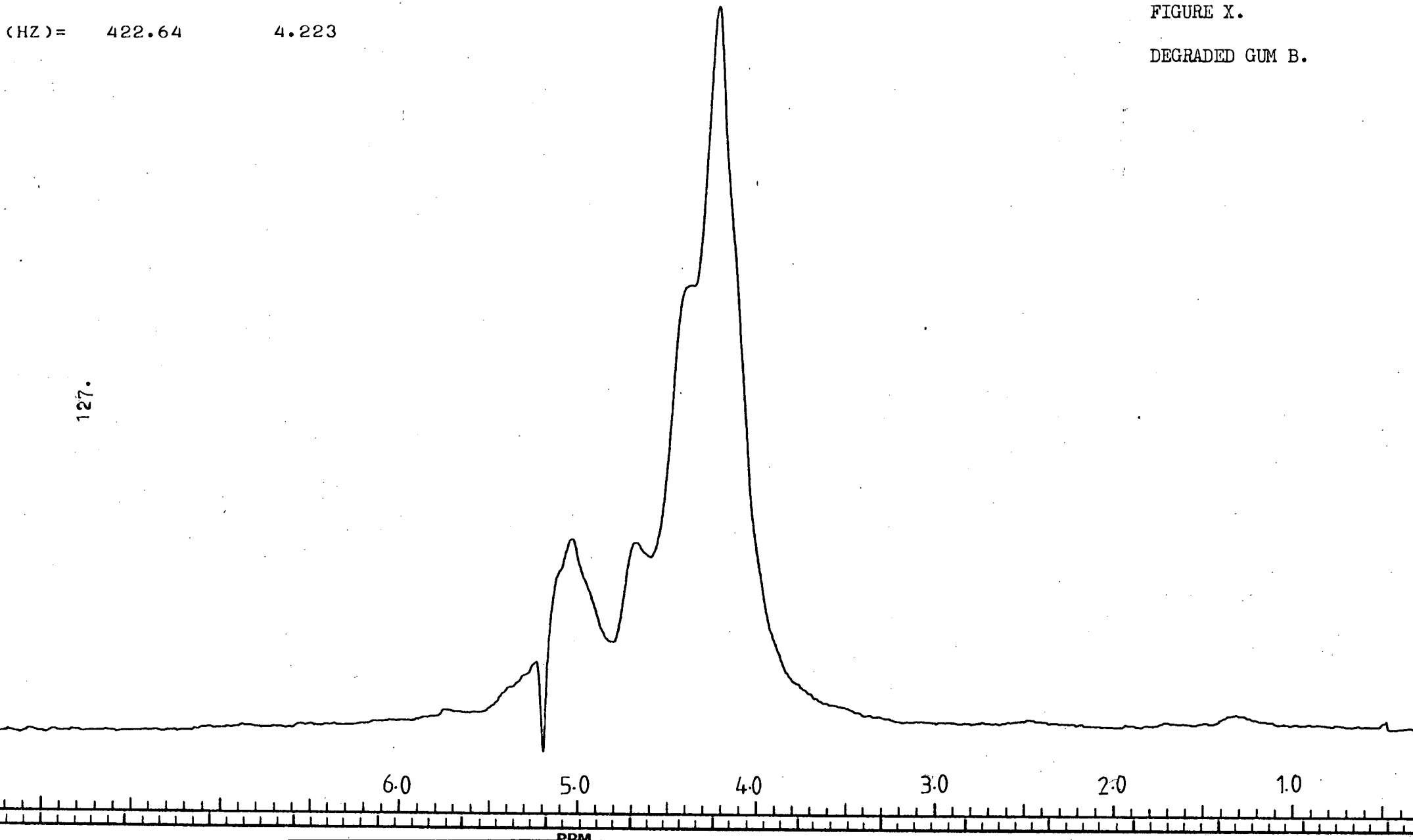
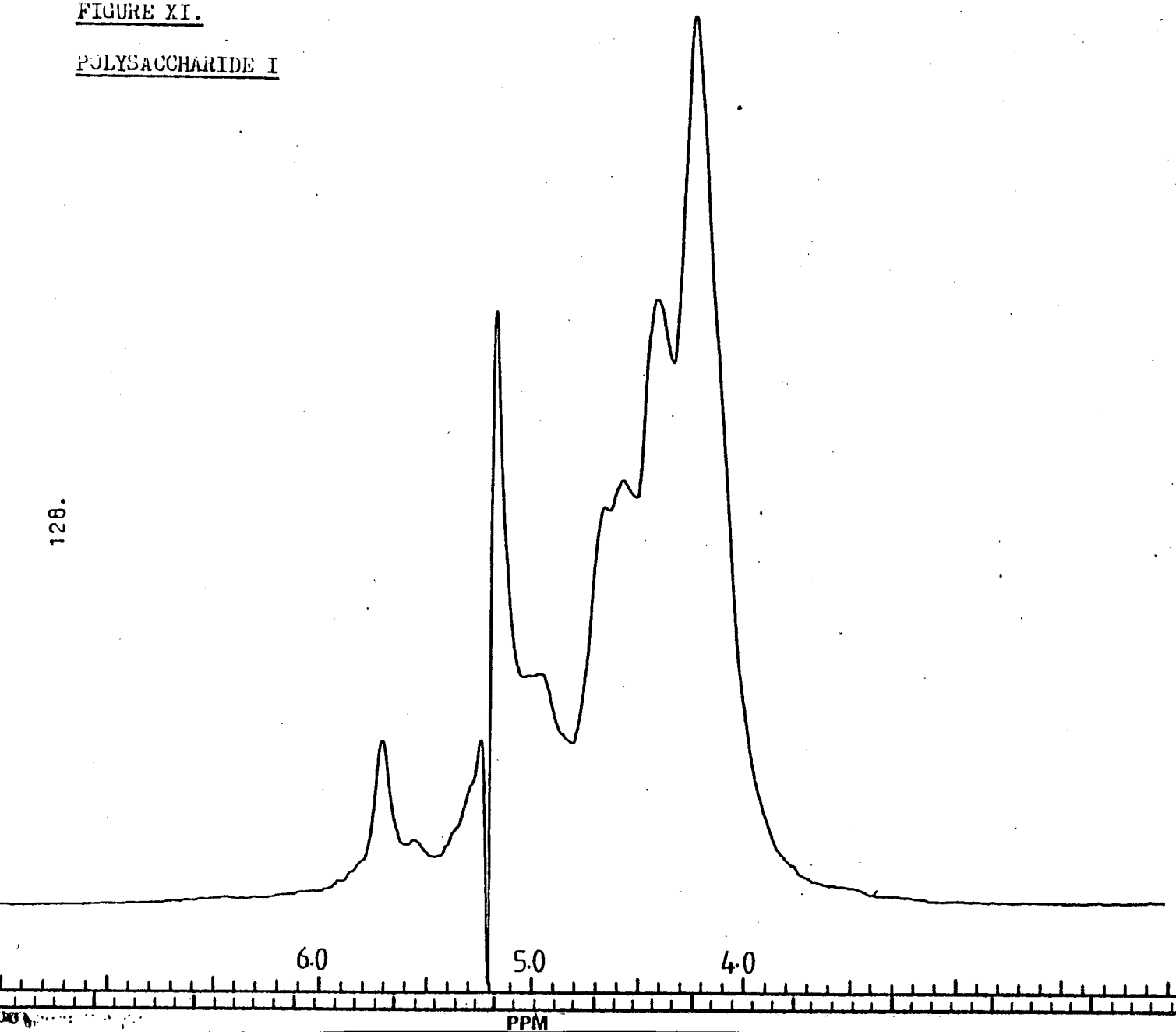


FIGURE XI.

POLYSACCHARIDE I



THRESH(CM)= 2.0
REF LN(HZ)= 12.00
RF FRQ(MHZ)= 100.0610

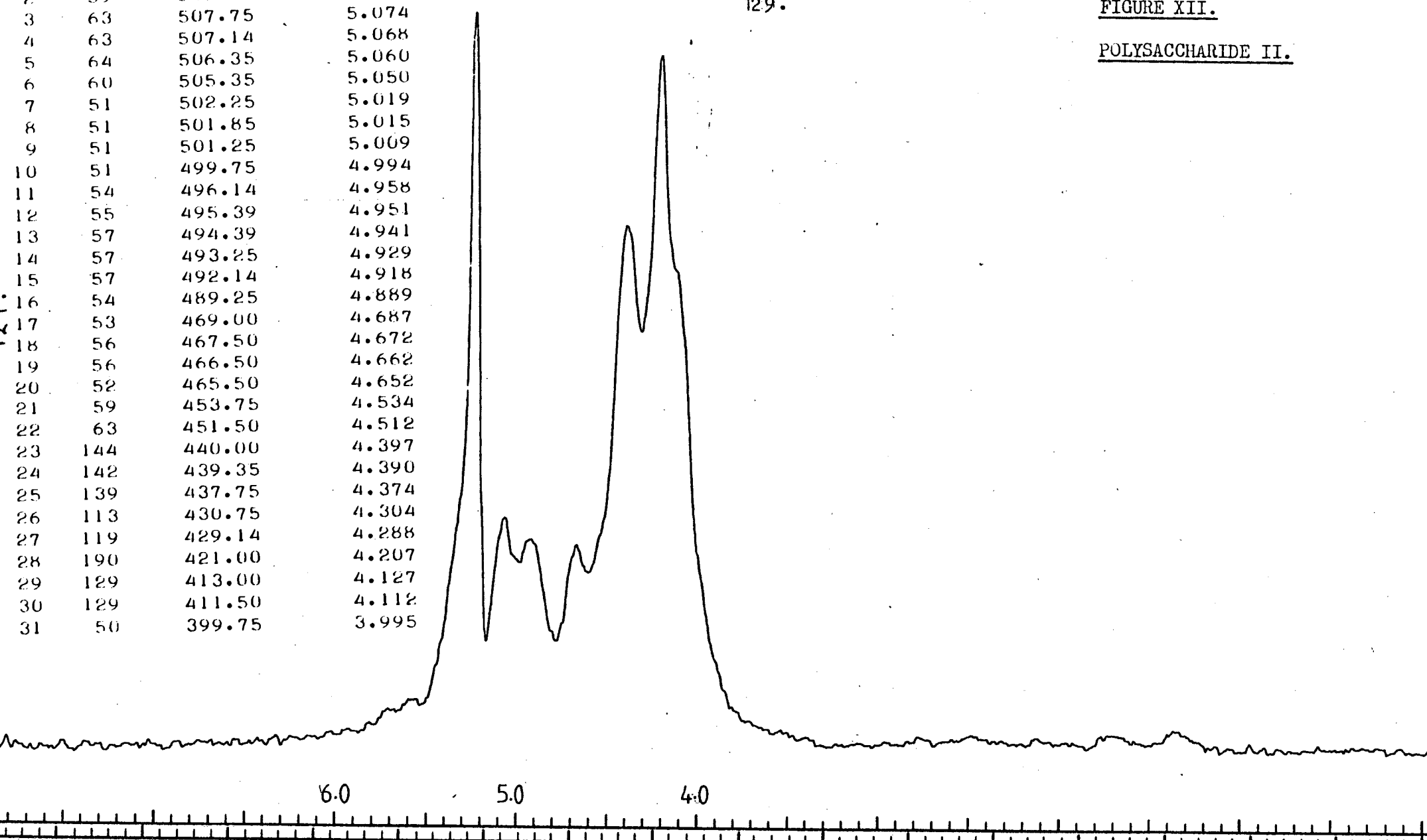
NO.	INT.	SHIFT(HZ)	PPM
1	38	570.60	5.702
2	38	570.10	5.697
3	28	528.00	5.276
4	38	523.75	5.234
5	139	516.25	5.159
6	50	504.75	5.044
7	50	504.25	5.039
8	51	503.39	5.031
9	52	502.39	5.021
10	52	502.00	5.017
11	52	501.60	5.013
12	52	501.10	5.008
13	52	499.50	4.992
14	52	499.00	4.987
15	52	498.50	4.982
16	52	497.75	4.974
17	53	497.00	4.967
18	52	495.75	4.954
19	37	485.35	4.850
20	37	484.85	4.845
21	36	483.85	4.835
22	36	483.00	4.827
23	36	482.35	4.820
24	35	481.75	4.814
25	35	481.00	4.807
26	92	466.00	4.657
27	92	465.50	4.652
28	89	463.85	4.635
29	92	461.64	4.613
30	98	457.50	4.572
31	93	453.00	4.527
32	92	452.50	4.522
33	92	452.00	4.517
34	92	451.50	4.512
35	92	451.00	4.507
36	141	441.00	4.407
37	207	422.00	4.217

FIGURE XII.

POLYSACCHARIDE II.

THRESH(CM)= 5.0
REF LN(HZ)= 12.00
REF FRQ(MHZ)= 100.0610

NO.	INT.	SHIFT(HZ)	PPM
1	202	523.50	5.231
2	59	509.00	5.087
3	63	507.75	5.074
4	63	507.14	5.068
5	64	506.35	5.060
6	60	505.35	5.050
7	51	502.25	5.019
8	51	501.85	5.015
9	51	501.25	5.009
10	51	499.75	4.994
11	54	496.14	4.958
12	55	495.39	4.951
13	57	494.39	4.941
14	57	493.25	4.929
15	57	492.14	4.918
16	54	489.25	4.889
17	53	469.00	4.687
18	56	467.50	4.672
19	56	466.50	4.662
20	52	465.50	4.652
21	59	453.75	4.534
22	63	451.50	4.512
23	144	440.00	4.397
24	142	439.35	4.390
25	139	437.75	4.374
26	113	430.75	4.304
27	119	429.14	4.288
28	190	421.00	4.207
29	129	413.00	4.127
30	129	411.50	4.112
31	50	399.75	3.995

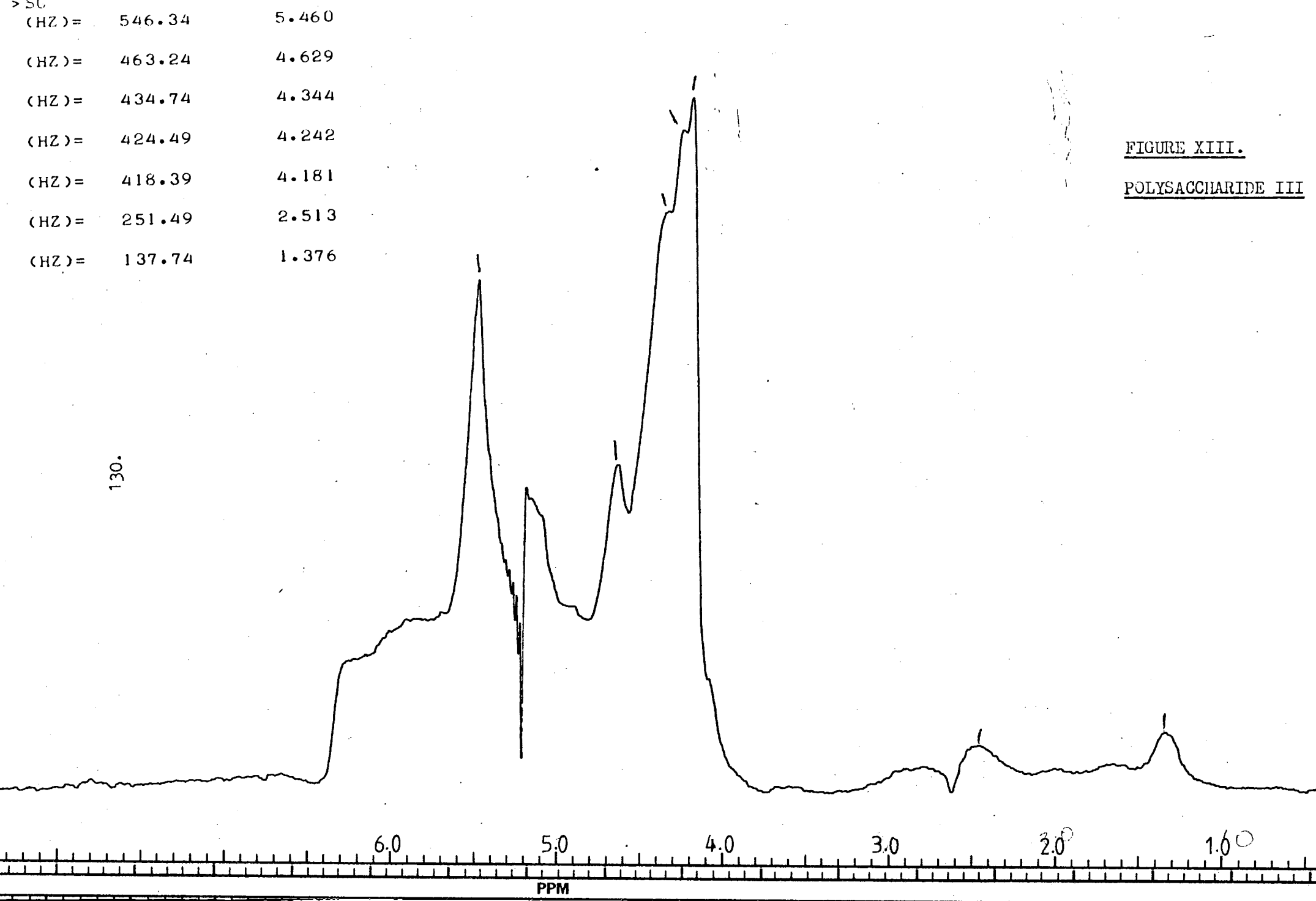


> SC

(HZ)=	546.34	5.460
(HZ)=	463.24	4.629
(HZ)=	434.74	4.344
(HZ)=	424.49	4.242
(HZ)=	418.39	4.181
(HZ)=	251.49	2.513
(HZ)=	137.74	1.376

FIGURE XIII.

POLYSACCHARIDE III



resonances (at 5.7, 5.6, 5.2, 5.1 and 4.8 p.p.m.) in the spectrum of Acacia xanthophloea gum (Fig. VIII) and degraded gum A (Fig. IX); these are probably due to the anomeric protons of 1,3 - linked galactose, 1,6 - linked galactose, the 1,6 - link between glucuronic acid and galactose, branched galactose residues and the anomeric protons of galactose-arabinose linkages. All of these linkages have been detected by chemical methods in Acacia xanthophloea gum (See Section VII). The resonances at 5.7 and 5.6 p.p.m. are probably due to 1,2 - and 1,3 - linked arabinose as these resonances almost disappear in the p.m.r. spectrum of degraded gum A (Fig. IX). The intensity of a resonance in the p.m.r. spectrum depends on its relative abundance in the molecule; degraded gum A contains much less arabinose than the whole gum. It was shown (Section VII) that most of the arabinose residues in A. xanthophloea gum are 1,2 - linked although some 1,3 - linkages are also present. The spectra of A. xanthophloea gum (Fig. VII) and degraded gum A (Fig. IX) both show the peak at 1.7 p.p.m. given by the CH_3 - protons of rhamnose. A. xanthophloea gum and degraded gum A were shown previously to contain rhamnose.

Degraded gum B is basically a galactan that remains after all the periodate-vulnerable sugar residues have been removed from degraded gum A. The p.m.r. spectrum (Fig. X) of degraded gum B is simpler than those of the previous spectra (Figs. VIII and IX) but it is still very complex; although the number of peaks due to the anomeric protons has decreased, one

broad peak still remains.

Polysaccharides I, II and III which were obtained from successive Smith-degradations from the whole gum (See Section VII) show complex p.m.r. spectra (Figs. XI, XII and XIII respectively). They all contain complex multiplet due to ring protons at 3.5 - 4.6 p.p.m. and the anomeric proton resonances at 4.6 - 5.7 p.p.m. Although the anomeric region is recognisable and distinct from the large complex multiplets, it is very difficult to make assignments, although the presence of an anomeric sign above 5.0 p.p.m. is indicative of hexose units linked in the β - configuration (4).

The p.m.r. spectrum shown by polysaccharide III, which is a galactan (See Section VII) is very complex. In addition to the two regions shown by polysaccharides I and II, there are broad peaks at 1.4 and 2.5 p.p.m. which probably arise from the proton atoms in the amino acids which make up the suspected protein or polypeptide part of the molecule (See Section VII).

Although p.m.r. spectroscopy has been used to determine the number of monosaccharide units in the repeating unit of some bacterial polysaccharides, the configuration of the anomeric centres and proportion of substituent groups (16), previous applications of p.m.r. to gum exudates has been limited. As the gum polysaccharides studied do not appear to have simple repeating units, it is difficult to assign peaks in the anomeric region. The spectra may possibly best be used as a method of detection and identification of some substituents e.g. the CH_3 - group of rhamnose, as a means of comparing gum polysaccharides to obtain indications of any structural similarities.

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